Ravindra Kumar · Anuja Gupta *Editors*

Seed-Borne Diseases of Agricultural Crops: Detection, Diagnosis & Management

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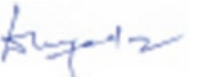
Foreword

The global population is increasing with great speed in recent times. To make food available for this ever-growing population is one of the greatest challenges facing scientists particularly agriculturalists around the world. Seed is one of the basic and key inputs in agriculture and its role will be very crucial in future to ensure global food security. More than 90% of all the crops cultivated worldwide are propagated by seed. Seed is also an efficient medium of harbouring and spreading of pathogens, which cause devastating diseases. The possibility of increasing cultivable land is negligible. Therefore, increasing production per unit area is the most appropriate option to sustain self-sufficiency in food. Hence, the demand for disease-free quality seeds and propagating material has been increasing exponentially in the last few decades.

Seed may harbour a variety of microflora, namely, fungi, bacteria, nematodes, viruses, etc., which can cause crop diseases and subsequently result in huge losses to agricultural crops. Therefore, seed-borne phytopathogens represent a major threat to the agriculture sector. The timely detection and diagnosis of seed-borne pathogens is essential in the adoption of effective management strategies. The idea of disease management does not aim at the complete control of the diseases but their management at economic threshold levels. This may be achieved with the implementation of the integrated disease management concept.

The book ***Seed-Borne Diseases of Agricultural Crops: Detection, Diagnosis & Management*** is a special effort to address the issues related to seed-borne diseases in different agricultural crops. This book addresses the importance of seed-borne

diseases; the diversity, detection and diagnosis of seed-borne phytopathogens; the management of seed-borne pathogens based on cultural, eco-friendly, chemical methods; and the integrated disease management concept. The book, in short, will explore and convey the key concepts and issues related to real-time situation of seed-borne/transmitted diseases. Experts in relevant fields have offered critical appraisal of various aspects and extended useful suggestions on seed-borne diseases, their detection, diagnosis and management. The contributors to this volume are all eminent authors actively engaged in research of their interest. This book is specially written for graduate and postgraduate level students of plant science, microbiology, plant pathology, seed pathology and seed sciences. In an era where the scenario of plant diseases is changing by the day, the efforts of **Dr. Ravindra Kumar and Dr. Anuja Gupta** in presenting this comprehensive volume to are truly commendable. I am quite sure that this book will be immensely useful to students, researchers and academicians, as well as to those involved in various agro-industries.



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February 15, 2019

D. K. Yadava

Preface

At present, the global population is already more than 7.0 billion, which will further increase to 11.2 billion in the year 2100, as estimated by the United Nations. To feed this ever-increasing population is a great challenge for agriculturalists around the world. Seed is one of the basic and prime inputs in the agriculture and its role will be very critical in the future to ensure global food security. About 90% of all the crops grown all over the world are propagated by seed. Seed is also an efficient medium of harbouring and spreading of pathogens, which cause devastating diseases. There is very little scope for increase in cultivable land. Therefore, increasing production per unit area is the most appropriate alternative to sustain self-sufficiency in food. Hence, demand for disease-free quality seed has been increasing exponentially in recent years.

Seed suffers from various types of diseases caused by a galaxy of microbes, namely, fungi, bacteria, nematodes, viruses, etc.; sometimes non-parasitic seed disorders caused by phytoplasma, viroids, etc. Seed-borne pathogens represent a major threat to crop establishment and yield. The seed-borne and seed transmitted diseases are of great economic significance especially in the tropical and subtropical developing countries. Excessive monoculture and faulty practices have resulted in the emergence of various plant diseases causing substantial economic losses. Hence, their timely detection and diagnosis is a must for their effective management. The concept of disease management does not aim at the complete control of the diseases but their management at economic threshold levels. Thus, integrated disease management requires understanding and integration of all aspects of crop production along with management strategies such as cultural practices, sanitation, eradication, soil amendments, biocontrol agents, resistant varieties, botanicals and judicious use of chemicals.

The book ***Seed-Borne Diseases of Agricultural Crops: Detection, Diagnosis & Management*** is an attempt to address the issues related to the seed-borne diseases in different agricultural crops. The book, covering over 25 chapters, is a comprehensive compilation of all issues that relate to the history of seed pathology, the importance of seed-borne diseases of agricultural crops, seed-borne diseases and quarantine, seed health testing and certification, detection and diagnosis of seed-borne diseases and their phytopathogens, host–parasite interactions during development of seed-borne diseases, diversity of seed-borne pathogens, seed-borne diseases of major agricultural crops, non-parasitic seed disorders, mechanisms of seed

transmission and seed infection, storage fungi and mycotoxins, impact of seed-borne diseases on human and animal health, and management options for seed-borne diseases. The book, in short, will explore and convey the key concepts and issues related to seed-borne/transmitted diseases.

The contributors of this volume are all eminent researchers actively engaged in the fields of their research. We thank all those who have contributed their valuable chapters to this book and we are sure that the present work written by learned experts will be immensely useful to students, researchers, academicians, progressive farmers, as well as those involved in various agro-industries. We hope that the available knowledge in this field will help in changing the present scenario of plant diseases.

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About the Editors

Ravindra Kumar is currently working as a Scientist Sr. Scale (Plant Pathology) at ICAR-Indian Agriculture Research Institute, Regional Station, Karnal-132 001 (Haryana). He received his B.Sc. (Agriculture) from CCS University, Meerut, and his M.Sc. (Agriculture) and Ph.D. (Ag.) from the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi, India. His areas of research interest are plant disease management, biological control, seed pathology, and plant microbe interaction. He has published more than 30 research papers and reviews in reputed national and international journals. He has 2 textbooks, 1 edited book, 5 book chapters, 8 technical bulletins, and more than 40 technical/popular articles to his credit. Dr. Kumar is also a life member of the Indian Phytopathological Society, New Delhi; Indian Society of Mycology and Plant Pathology, Udaipur; and Indian Society of Seed Technology, New Delhi.

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Part I

Impact of Seed-Borne Phytopathogens or Seed-Borne Diseases on Agriculture and Society

1 Importance of Seed-Borne Diseases of Agricultural Crops: Economic Losses and Impact on Society

Ashok Gaur, Atul Kumar, Raj Kiran, and Pooja Kumari

Abstract

To satisfy the basic nutritional needs of growing population of the world, there is an urgent need to increase in agricultural production. As majority of crop is propagated by seed, planting quality healthy seed is an important factor. Major seed quality characters are physical quality, genetic purity, physiological quality and seed health. The health status of seed is judged by presence or absence of insect infestation and seed-borne diseases. Seed-borne refers to the particular plant diseases that are transmitted by seed. Seed which is free from disease inoculum is a primary means of reducing the introduction of pathogens into fields, and it also reduces inoculum production and the efficacy of secondary spread to the inoculum threshold and disease severity after establishment. The quality of seeds alone is known to account for an increase in productivity of at least 10–15%. To achieve this high quality, all the factors in production that will affect seed viability, disease-free and genetic purity should be taken into account. The success of modern agriculture depends on pathogen-free seed with high yielding character and in turn disease management. There were many epidemics which were seen during the early 1990s, but with advancement of new management practices and use of new technologies in seed treatment, it helped in bringing down the epidemics due to plant diseases.

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1.1 What Is Disease

Man depends upon plants for his very existence. Only green plants and some blue-green algae can convert solar energy into food. So, man and other animals exist on earth as guests of the plant kingdom. Three-fourths of the total world food supply is drawn from the grasses, and the human civilization rests on the cultivation of cereal grains.

Plant pathologists study the causes and development of plant diseases and try to develop practical management strategies. These studies are known as plant pathology or phytopathology. These words have been derived from Greek words *Phyto, Pathos* and *Logos* which means plant, disease and study, respectively. In fact, everybody knows that literally the term disease stands for dis-ease, i.e. not at ease. The whole plant or any of its part may be diseased, and this may happen at any time during the life cycle of the plant.

A number of definitions have been proposed from time to time by various pathologists, some of them are given below:

1. The oldest Indian scripture on medicine is *Sushruta Samhita* which dates back to 2000 BC. In this scripture it is stated that 'anything that afflicts the inner man (self or *Purusha*) is disease and disease has its primary seat in the inner spring of vitality from which it flows out to the surface, to the external body'.
2. Almost similar views are expressed by Hahnemann (1810), the Father of Homeopathy. According to him the disease is when a person falls ill, it is only this spiritual self-acting vital force, everywhere present in the organism, that is primarily deranged by the dynamic influence or a morbific agent inimical to life-orgenon.
3. Kuhn (1858) said that the diseases of plant are to be attributed to abnormal changes in their physiological processes; they are disturbances in normal activity of their organs.
4. According to Ward (1896), it is a condition in which the functions of the organism are improperly discharged, or in other words, it is a state which is physiologically abnormal and threatens the life of the being or organ.
5. Hartig (1900) said that I do not regard investigations of mere sickness as a task of pathology. It is only when sickly condition leads to the death of some part of the plant that we speak of actual disease.
6. According to Freeman (1905), when favourable conditions of life are so seriously interfered by any agency that life as part of plant or whole plant is threatened, we recognize disease in plants.
7. According to Butler (1918), disease in plant is a variation from normal physiological activity, which is sufficiently permanent or extensive to check the performance of plant's natural function or completion of its development.
8. According to the American Phytopathological Society (1940), disease is a deviation from normal functioning of physiological processes of sufficient duration or intensity to cause disturbance or cessation of vital activity.

9. According to Heald (1943), disease is any alteration of the normal state of plant or some of its organs or parts interrupting or disturbing the performance of vital functions or any departure from the state of health presenting marked symptoms or injurious effect.
10. According to the British Mycological Society (1950), disease is a harmful deviation from the normal functioning of process.
11. According to Stakman and Harrar (1957), a plant disease is a physiological disorder or structural abnormality that is harmful to the plant or any of its products or that reduces its economic value.
12. According to Walker (1957), diseased plants are those which have become altered in their physiological and morphological development to such a degree that signs of such effects are obvious.
13. According to Horsfall and Dimond (1959), disease:
    (i) Is not a pathogen but it is caused by a pathogen.
    (ii) Is not the symptoms but results in symptoms.
    (iii) Is not a condition as the condition results from disease and is not synonymous with it.
    (iv) Is not an injury.
    (v) Cannot be catching or infectious; it is actually the pathogen which is catching and infectious.
    (vi) Results from continuous irritation.
    (vii) Is a malfunctioning process, and this must result in some suffering and hence disease is a pathological process.
14. Kent (1973) said that disease is the diminution or loss of the ability to correlate the utilization of energy within an individual as a result of continued irritation of pathogen.
15. According to Wheeler (1975), disease is when all malfunctions which result in unsatisfactory plant performance or which reduces a plant's ability to survive and maintain its ecological niche.
16. According to Robinson (1976), disease is an adverse effect on a host due to pathogen.
17. Dasgupta (1977) defined disease as a malfunctioning process involving more or less continuous interaction between host and pathogen occurring through a large number of quantitative changes between qualitative states of healthy and diseased condition.
18. According to Bateman (1978), disease is the injurious alteration of one or more ordered processes of energy utilization in living system caused by the continued irritation of a primary causal factor or factors. When a living system is altered beyond range of its easy tolerance, it is diseased as opposed to healthy. If the system is pushed beyond its limit of absolute tolerance, it will die.
19. According to Encyclopedia Americana, disease is the impairment of the health or normal functioning of an organism. Disease may primarily affect a single organ or tissue or a group of organs or tissues or the entire body. They may result from wide variety of causes, including infectious organisms, hereditary factors and environmental hazards.

20. As per Encyclopaedia Britannica, disease is a departure from the normal physiological states of a living organism sufficient to produce overt signs or symptoms.
21. In Webster's Third New International Dictionary, disease is an impairment of the normal state of the living animal or plant body or any of its components that interrupts or modifies the performance of the vital functions, being a response to the environmental factors, to specific infective agents, to inherent defects of the organism or to combination of these factors.
22. According to modern concept, disease is an interaction between host, parasite and the environment.

All the above-mentioned definitions indicate that disease:

(a) Reduces the ability of the plant to survive
(b) Is related to poor functioning of growth and reproduction
(c) Results in malfunctioning of physiological activities

Keeping in mind the various views, Singh (1989) proposed the following definition of the disease:

> A sum total of the altered and induced biochemical reactions in a system of a plant or plant parts brought about by any biotic or abiotic factor(s) or a virus leading to malfunctioning of its physiological processes, and ultimately manifesting gradually at cellular and/or morphological level. All these alterations should be of such a magnitude that they become a threat to the normal growth and reproduction of the plant.

As per this definition plant disease includes all the abnormalities in plants which are mediated by changes in their metabolism and whose development is gradual irrespective of cause or nature of association. However, wilting, drying or burning of plant or its parts, which is sudden as in case of fire, high dosage of a herbicide on sensitive plants or very high intensity of heat or cold should be considered as injury.

1.2 What Are Seed-Borne Diseases?

Plant pathologists normally divide the plant diseases, for the convenience of study, into three categories:

1. Seed-borne diseases
2. Soil-borne diseases
3. Air-borne diseases

There is no sharp line of demarcation between these three categories and a pathogen may adopt one or more methods for its survival, for example, loose smut of wheat caused by *Ustilago segetum* var. *tritici* is an exclusively internally seed-borne and seed-transmitted disease, as the dormant mycelium of the pathogen is located in the

embryo of the seed. When these infected seeds are sown, the mycelium becomes active and grows with the host plant, silently, without producing any symptom. When after flowering the ear-heads are produced, then the pathogen expresses itself and instead of normal ear-head with healthy grains, the smutted ear-head is produced containing millions of chlamydospores of the pathogen. Now, these chlamydospores are blown away by wind to cause infection to another healthy flower. Thus, we see that though the disease is seed-borne yet it takes the help of wind to complete its life cycle. So it is the primary initiation of infection which decides the nature of the disease. However, here we will restrict ourselves only to seed-borne diseases. The study of seed-borne diseases is known as Seed Pathology.

The term *Seed Pathology* was first used by Paul Neergaard and Mary Noble in 1940, though the science of Seed Pathology was known to exist for more than 100 years. From the early 1960s, Dr. Paul Neergaard not only emphasized the importance of seed-borne diseases but also established an institute which was known as Danish Government Institute of Seed Pathology for Developing Countries (DGIPS) in Copenhagen, Denmark, in April 1967. He was the founder director of the institute and continued at the institute till his retirement in 1982.

Seeds are the victims and the vehicle of small propagules of the pathogens which are carried by the seed from one generation to the next generation. Seeds are also regarded as an integral component of world trade which is destined to be consumed either as food or used to grow a crop. According to Schwinn (1994), of all the food crops grown in the world, about 90% are propagated through seeds. With the frequent and increased movement of seed from one part of the world to another or from one corner to another corner of the same country, seed becomes a silent carrier of the pathogens which are transmitted when the seeds are sown to grow a particular crop. Fungi, bacteria, viruses and even nematodes are carried in the seed, on the seed or with the seed. Accordingly, the pathogens are known as:

1. Externally seed-borne pathogens
2. Internally seed-borne pathogens
3. Concomitant contaminants

It is interesting to note that a pathogen may be seed-borne yet not seed-transmitted because the establishment of infection in seedling and in adult plant is the last decisive link in the process of seed transmission. Moreover, the development of a seed-borne disease is a complicated process resulting from the interaction of pathogen, saprophytes present in the soil and leachates produced by the seeds when they are sown. The uredospores of *Puccinia carthemi* Corda causing rust in safflower remains viable for a short duration and so are unable to secure transmission, but its teleutospores survive for a longer duration and are significant for the transmission of rust in safflower. Similarly, the blind disease caused by *Gloeotinia temulenta* (Prill & Delacr.) Wilson, Noble and Gray in *Lolium perenne* Linn. can be transmitted through the mycelium adhering to the seed surface but not through macroconidia, because mycelium can remain viable up to 2 years while macroconidia can survive only for 2 months.

1.2.1 Externally Seed-Borne Diseases

When the pathogen is present on the surface of the seed in the form of conidia, mycelium or any other kind of resting spore, then the pathogen is known as externally seed-borne pathogen and the disease caused by it, as externally seed-borne disease, e.g. smut spores of *Tolyposporium penicillariae* causing smut of pearl millet adhering to the seed surface are externally seed-borne.

1.2.2 Internally Seed-Borne Diseases

When the pathogen is present anywhere inside the seed, the pathogen is known as internally seed-borne pathogen and the disease as internally seed-borne disease. Inside the seed the pathogen may be present either just below the seed-coat or the endosperm or the embryo. Most of the seed-transmitted members of the class *Deuteromycetes* are located in the seed-coat. Normally they establish themselves as dormant mycelium which resumes its activity as and when the suitable environmental conditions are available, e.g. the dormant mycelium of *Botrytis anthophila* causing the blossom blight of red clover is present in the seed-coat (Bennum 1972). As the seed germinates, the mycelium becomes active and starts growing into the root through hypocotyl. The mycelium grows intercellularly into all parts of the host. However, it does not enter into the vascular system. At the time of flowering the mycelium enters into the young flowers and penetrates the anthers destroying the pollen grains.

1.2.3 Admixture or Concomitant Contaminants

When the pathogen is present along with the seed as a contamination, then it is known as concomitant contaminant. Here the pathogen is present in the form of a fruiting body of the pathogen like sclerotia; e.g. the sclerotia of *Claviceps fusiformis* Loveless, the causal organism of ergot of pearl millet, is just mixed up with the seeds which can be separated mechanically, either by winnowing or sieving out the sclerotia from the brine solution or by using the gravity separator (Pathak et al. 1984).

1.3 Importance of Seed-Borne Diseases

Importance of seed-borne disease can be seen or observed by the types of losses it causes. The impact of fungi on seed is considerable.

1.3.1 Reduction in Crop Yield

There are both qualitative as well as quantitative losses due to seed-borne diseases. The reduction in crop yields may occur due to many types of abnormalities.

(a) **Seed abortion:** It is seen mostly in smut and ergot fungi where flower organs of the hosts are replaced by the fructifications of the parasites, e.g. *Ustilago* sp. and *Claviceps* sp.
(b) **Shrunken seeds, reduced seed size:** It is seen in crucifers infected by *Alternaria brassicicola* and *Phoma lingam*.
(c) **Seed rot:** Many seed-borne fungi cause rots either in crop or during germination; e.g. *Fusarium avenaceum* and *Drechslera* sp. in cereals cause seed rot.
(d) **Sclerotization/stomatization of seed:** Transformation of floral organs or seed into sclerotia or stromata is an important disease condition in certain categories of fungi and host, e.g. ergot produced by *Claviceps purpurea*.
(e) **Seed necrosis:** Many seed-rotting fungi produce superficial necroses in the seed. Most seed-borne fungi usually do not go beyond the protective layer of the seed coat or pericarp. In leguminous seeds, anthracnose fungi *Colletotrichum* sp. as well as *Ascochyta* sp. often penetrate into the fleshy cotyledons producing conspicuous necrotic lesions in seeds of bean, soybean, pea, chickpea, cowpea, etc.
(f) **Seed discolouration:** It is a very important degrading factor. It can be categorized broadly into three types, namely:
    1. Superficial necrotic lesions: Many seed-borne parasitic fungi infect the seed coat causing conspicuous necrotic black-brown to grey discolouration, e.g. *Ascochyta pisi* in pea, *Colletotrichum lindemuthianum* in beans and *Drechslera oryzae* in rice (pecky rice).
    2. Fungus coating: It is seen in black point/kernel smudge in cereals. Profuse growth is observed in *Drechslera sorokiniana* in wheat, *Drechslera oryzae* in paddy and *Drechslera teres* in barley.
    3. Pigments: Different colours of pigments are observed in different pathogens, e.g. purple stain in soybean by *Cercospora kikuchii*, pink discolouration seeds of maize by *Fusarium moniliforme*, red discolouration in beans also called red nose by *Stemphylium botryosum,* pink stain in rice by *Trichoconiella padwickii* and blue stain in cotton lint by *Alternaria tenuis*.
(g) **Reduction or elimination of germination capacity/lowered viability:** It is seen in many wheat and barley diseases caused by smut fungi (Agarwal and Sinclair 1997).

1.3.2 Loss in Germination and Vigour

Many seed-borne pathogens become active when seeds are sown, which may result in seed decay and/or pre- or post-emergence damping off. This in turn results in

poor plant stand in the field. Some examples of diseases which lead to loss in germination and vigour are listed below.

1. Stack burn (*Alternaria padwickii*) causes decay of rice seeds, roots and coleoptiles, resulting in the death of young seedlings.
2. *Fusarium moniliforme* (foot rot/bakanae) causes seed rot, seedling blight and brown discolouration in the coleoptile and in primary and secondary leaves of rice.
3. *Bipolaris oryzae* (brown spot)-infected rice seeds have been found to be lower in germination than healthy seeds.
4. *Neovossia indica* (karnal bunt)-infected wheat seeds have a significantly lower survival rate than healthy seeds.
5. *Colletotrichum truncatum* (anthracnose), *Drechslera phaseolorum* var. *sojae* (stem canker) and *Sclerotinia sclerotiorum* (sclerotinia stem rot) which are all internally seed-borne diseases of soybean show poor field emergence.
6. *Cercospora kikuchii* causing purple stain in soybean showed 12% reduction in seedling emergence and *Macrophomina phaseolina* causing charcoal rot showed decrease in seedling emergence by 59%.
7. *Phytophthora nicotianae* var. *parasitica* which causes fruit rot in tomato also resulted in poor germination. It was observed that seeds either failed to germinate, or if they germinated, seedlings were killed by the fungus. Similar observations were seen in barley seeds severely infected with spot blotch caused by *Bipolaris sorokiniana* which did not germinate, or if they germinated, seedlings became infected.
8. *Diplodia maydis* which causes stalk rot/white ear rot/root rot in maize also caused reduction in maize seed germination.

In some bacterial pathogens like *Corynebacterium michiganensis* pv. *michiganensis* in pepper and *Pseudomonas syringae* pv. *glycinea* in soybean, loss in germination and vigour have been observed in seeds. About 31–35% reduction in germination was reported in alfalfa seeds due to infection by alfalfa mosaic virus, and many a times virus-infected seeds may even fail to germinate (Agarwal and Sinclair 1997).

1.3.3 Development of Plant Diseases

A pathogen can be distributed to new areas through seeds. Plant pathogens have spread with planting material since crop cultivation began. Before modern times, the movement of pathogens was restricted to certain localized areas because of limited means of travel. However, today, with expanded and rapid modes of transport, chances of spread through seeds have increased to include large areas within a country and from one country to another. The exchange of plant material between countries has enhanced the risks of introducing new pathogens with seeds. Thus, new

strains or physiologic races of a pathogen may be introduced with new germ plasm from other countries.

Seeds are the most important means for perpetuation of plant pathogens. For certain pathogens, such as those that cause loose smut of wheat, covered smut of barley and barley stripe mosaic disease in barley and wheat, seeds are the exclusive means of survival. The role of such inocula in disease establishment in the field is well documented. Transmission of a pathogen through seeds is considered more important than other survival means. The pathogens remain viable longer in seeds than in vegetative plant parts or in soil. The host-parasite relationship within seeds also favours the earliest possible infection in the field. Since pathogens are in direct contact with the seeds, the chances of seedling infection are enhanced. Thus, seed-borne infection can provide a focus for inoculum, which may spread under favourable conditions and cause an epidemic.

A plant disease can develop into an epiphytotic condition, if the infected seeds are present in the seed lot used for sowing in large areas. For example, black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris* even if the infection is 0.02%, it can result in epiphytotic condition. Similarly, in other bacterial pathogens like *Xanthomonas campestris* pv. *phaseoli* in bean infection of 0.5% and *Xanthomonas campestris* pv. *vesicatoria* if the infection is even less than 1%, it can result into an epiphytotic condition. Teliospores of *Tilletia controversa*, *T. laevis* and *T. caries* can be disseminated widely by importation through the use of contaminated seeds, animal-derived fertilizers and natural movement of seed-eating migratory animals. It is common practice to feed severely contaminated seed to animals. The mechanical application of manure from feedlots as fertilizer could deposit teliospores on the soil surface and provide a source of infection for wheat (Agarwal and Sinclair 1997).

1.3.4 Discolouration and Shriveling

Discolouration can indicate undesirable physical qualities, the presence of toxic metabolites or other unfavourable seed characteristics. Discolouration on soybean seeds, caused by various microorganisms, which have long been used for diagnostic purposes, can indicate seed quality.

1. Soybean seeds infected with *Phomopsis longicolla* are elongated, smaller than normal, deeply fissured and covered with whitish mycelium.
2. Dull-grey to deep-brown patches and scattered, dark sunken areas on a soybean seed are due to infection by *Alternaria alternata* and *A. tenuissima.*
3. Salmon or pink to red discolouration anywhere on a soybean seed coat may be associated with infection by *Fusarium graminearum* and *F. sporotrichioides*.
4. Irregular brown or grey areas with black specks develop on soybean seed coats infected with *Colletotrichum truncatum.*
5. Purple stain caused by *Cercospora kikuchii* can vary from violet to pale purple to dark purple, and the colour tends to bleed from the hilum.

6. Seeds from a soybean plant with downy mildew become encrusted with oospores of *Peronospora manshurica*.
7. Seeds from soybean plants with soybean mosaic virus are mottled with brown or black, depending on hilum colour.
8. Soybean seeds infected with *Cercospora sojina* develop conspicuous light- to dark-grey or brown areas that vary from minute specks to large blotches covering the entire seed coat. Some lesions show alternating bands of light- and dark-brown colour. Occasionally, brown and grey lesions diffuse into each other. Normally the seed coat cracks.
9. Symptoms on soybean seeds infected by *Macrophomina phaseolina* appear as indefinite black spots or blemishes on the seed coat.
10. Soybean seeds colonized with *F. oxysporum* appear shrunken, slightly irregular in shape, often with cracks in the seed coat with light- to dark-pink discoloured areas over most of the seed surface.

Some pathogens that cause discolouration in soybean seeds affect only seed coat colour, causing "cosmetic" or "superficial" damage, while other pathogens damage tissue in the seed coat and embryo.

1. Chickpea seeds infected with *Phoma rabiei* are small and wrinkled, with dark-brown lesions of various shapes and sizes with pycnidia found in deep lesions.
2. Pea seeds infected with *M. pinodes* have dark-brown lesions.
3. A number of fungi, such as *Alternaria alternata*, *A. padwickii, Curvularia lunata* and *Bipolaris oryzae* induce a brown discolouration on rice seeds.
4. *Ascochyta fabae* causes various degrees of discolouration on faba bean, depending on infection levels.
5. *Pseudomonas glumae* causes seed discolouration and formation of brown bands across the endosperm in rice.
6. *C. michiganensis* pv. *michiganensis* in pepper show brown discolouration and infected seeds are smaller than healthy seeds.
7. Bean seeds infected with *Colletotrichum lindemuthianum* showed brown to light chocolate-coloured, sunken cankers on seed coats. Lesions may extend into the cotyledons following early infection. Less severely infected seeds showed yellowish to brown sunken lesions, which were not always distinguishable from those caused by other organisms.
8. *Ascochyta fabae* f. sp. *lentis*-infected lentil seeds were shriveled and discoloured, and seed quality was reduced. Reduced seed size was correlated significantly to the level of seed-borne inoculum (Agarwal and Sinclair 1997).

1.3.5 Biochemical Changes in Seeds

Many seed-borne fungi induce qualitative changes in the physicochemical properties of seeds, such as colour, odour, oil content, iodine and saponification value, refractive index and protein content, thereby affecting their commercial value.

Biochemical deterioration and change in the quality of seed nutrients due to different pathogens have been observed.

1.3.5.1 Protein

Phomopsis is more important than *Fusarium* in soybean seed deterioration; flour and oil derived from seeds infected with *Phomopsis* were unmarketable. Soybean seeds infected by *Fusarium* or *Phomopsis* have lower-quality oil, higher amounts of free fatty acids, poor meal colour and other reduced quality factors when compared to noninfected seeds. *C. kikuchii* colonizing the entire soybean seed surface tends to lower oil and increase protein content. In soybean seeds encrusted with oospores of *P. manshurica*, the protein content and free fatty acid content was higher and oil content lower than in healthy seeds. Infection by *Alternaria padwickii* resulted in a reduction of total carbohydrates in rice. Infected seeds had 65.7% carbohydrates, compared to 76.7% in healthy seeds. Infection of peanut seeds by *Aspergillus flavus, A. niger* and *R. solani* increased crude fat content but reduced starch content, probably due to amylase production by these fungi. *Macrophomina phaseolina* infection in peanut caused both qualitative and quantitative damage, including discolouration of pods and seeds, and a reduction in pod yield, seed yield and oil content. In barley seeds, protein increased as the proportion of seed infected by barley stripe mosaic virus increased. High protein content in malting barley is undesirable.

1.3.5.2 Oil

The chemical changes resulting in increased unsaturated fatty acids are undesirable and can cause obesity and cardiovascular disorders in humans. Hence, such seeds are not fit for oil extraction and human consumption or for derived oil cake as animal feed. Oil is synthesized in seeds by condensation of molecules of glycerine or fatty acids, or both. The glycerine and fatty acids are synthesized from carbohydrate, especially reducing sugars. Naturally occurring mixtures of glycerides in oil contain a small amount of free fatty acids. The increase in free fatty acids shows that fungi have a high lipase activity, which can bring about fat hydrolysis.

The oil content in sunflower seeds is reduced due to infection of *M. phaseolina*. The oil colour from inoculated seeds changes from light yellow to yellowish brown, and the free fatty acid content increases. The I-number indicates the quantity of unsaturated acids present in oil. Vegetable oils are high in unsaturated fatty acids. The decreased I-value observed in *Fusarium*-infected canola oil was indicative of decreased unsaturation created by the metabolic activity of the microbes. Decreased I-value due to fungal infection also has been reported in mustard, peanut, sunflower and sesame oil.

The oil content of infected seeds may be reduced. The oil content of peanut seeds was reduced by infection of many *Aspergillus* sp., *Botryodiplodia* sp. and *Cladosporium herbarum.* A high percentage of free fatty acids showed that fungi caused a hydrolytic type of deterioration in oil, which results in oil rancidity. A rancid taste in peanut oil due to free fatty acids was reported (Agarwal and Sinclair 1997).

1.3.5.3 Toxin

Many fungi produce toxins in seeds which makes them unsuitable for consumption as feed. Aflatoxin in peanut is produced by infection of *Aspergillus flavus* which is harmful to animals, if they feed on such infected peanuts. Rice grains infected with *Penicillium islandicum* become yellow and animals feeding on it show extensive liver damage causing death. *Gibberella zeae*-infected maize grains caused hypertrophy in swine. *Claviceps purpurea*-infected grains lead to abortion of cattle if they feed on such grains.

1.3.6 Alteration in Physical Properties of Seeds

Physical properties such as density, shape, size, surface, volume and weight are economically important and are affected by many fungal species such as *Alternaria, Fusarium* and *Phomopsis* in soybean. *Phomopsis* reduced seed density by 4% and volume and weight by 13%, with a resultant potential of seed breakage 20 times greater than that for asymptomatic seeds. All types of fungal damage are treated equally, regardless of source or type. Infection by *Curvularia lunata* and *Fusarium moniliforme* significantly reduced the starch granule size in infected sorghum seeds compared to infected seeds. The reduction in size increased with increase in infection severity. The infection of seeds by *F. moniliforme* also resulted in pitting on starch granule surfaces (Agarwal and Sinclair 1997).

1.4 Economic Losses and Impact on Society

Crop losses due to seed-borne diseases are commonly displayed in the form of disastrous effects of plant disease epidemics. Whether long or short, localized or widespread, weak or massive spread of diseases, all poses a quite different outcomes with different dimensions. For example, various seed-borne diseases cause wide range of yield losses in rice crop (Table 1.1).

With the advancement of new plant disease management practices, like availability of seeds of new variety which are host resistant and tolerant, more effective seed treating fungicides and biocontrol agents and integrated pest management, all these have reduced the frequency of disease epidemics in recent years.

Table 1.1 One of the examples of chronic yield losses by harmful organisms in rice in tropical Asia (Savary et al. 2000)

Seed-borne diseases	Percent yield loss
Bacterial blight of rice	0–0.6
Sheath blight	5–10
Brown spot	0–10
Leaf blast	0–1.7
Neck blast	0–2.1
Sheath rot	1.3–7.3

A comprehensive list of various seed-borne diseases caused by fungi, bacteria, nematodes and viruses as mentioned by Gaur (2011) is being given in Tables 1.2, 1.3, 1.4 and 1.5.

1.5 Conclusion

The high-quality healthy seeds are necessary for first level of disease management. The most important prerequisite for good crop production is the availability of good quality disease-free seeds. Seed-borne diseases are of three types; it may be internally seed-borne, externally seed-borne or as admixture, as management practices depend upon the place of presence of pathogen's inoculum in the seed. The direct losses caused by the seed-borne diseases can be measured by reduction in yield, loss in germination and vigour, development of plant diseases, shriveling, discolouration and biochemical changes in the seed. These seed-borne diseases played an impact on society in the form of different epidemics at different time, but because of adoption of new technologies in the modern agriculture, occurrence of epidemics has been reduced.

Table 1.2 Important seed-borne fungal diseases of cereals, vegetables and oil-seed crops

S. No.	Crop	Disease	Causal organism
1.	Barley	Ergot	*Claviceps purpurea*
		Seedling blight, root rot	*Bipolaris sorokiniana*
		Scab, seedling blight	*Fusarium graminearum*
		Snow mould, brown foot rot	*Microdochium nivale*
		Glume blotch	*Stagonospora nodorum*
		Leaf stripe	*Drechslera graminea*
		Net blotch	*Drechslera teres*
		Covered smut	*Ustilago segetum* var. *segetum*
		Loose smut	*Ustilago segetum* var. *nuda*
2.	Bean	Anthracnose	*Colletotrichum lindemuthianum*
		Blight	*Diaporthe phaseolorum*
		Pod and stem blight	*Diaporthe phaseolorum* var. *sojae*
		Wilt	*Fusarium oxysporum* f. sp. *phaseoli*
		Charcoal rot	*Macrophomina phaseolina*
		Collar rot	*Phytophthora phaseoli*
		Damping off, stem canker	*Rhizoctonia solani*
		Wilt, stem rot	*Sclerotinia sclerotiorum*

(continued)

Table 1.2 (continued)

S. No.	Crop	Disease	Causal organism
3.	Brassica	White blister	*Alubugo candida*
		Grey leaf spot	*Alternaria brassicae*
		Black spot	*A. Brassicicola*
		Grey mould	*Botrytis cinerea*
		Downy mildew	*Peronospora parasitica*
		Black leg	*Phoma lingam*
		Watery soft rot	*Sclerotinia sclerotiorum*
4.	Brinjal	Fruit rot	*Phytophthora vexans*
		Wilt	*Verticillium alboatrum*
5.	Carrot	Leaf blight	*Alternaria dauci*
		Black root rot	*Alerternaria radicina*
6.	Caster bean	Capsule mould, seedling blight	*Alternaria ricini*
7.	Celery	Root rot, black neck	*Phoma apiicola*
		Late blight, leaf spot	*Septoria apiicola*
8.	Cotton	Leaf spot	*Alternaria macrospora*
		Seedling blight	*Ascochyta gossypii*
		Anthracnose, seedling blight, bole rot	*Colletotrichum gloeosporioides*
		Wilt	*Fusarium oxysporum* f. sp. *vasinfectum*
		Foot rot, stem blight	*Macrophomina phaseolina*
		Damping off, seedling blight	*Rhizoctonia solani*
		Wilt	*Verticillium alboatrum*
9.	Cluster bean	Blight	*Alternaria cyamopsidis*
10.	Cowpea	Ashy stem blight	*Macrophomina phaseolina*
11.	Chilli	Ripe rot, anthracnose	*Colletotrichum capsici*
		Brown spot	*Ralstonia solanacearum*
12.	Coriander	Stem gall	*Protomyces macrosporus*
13.	Cucumber	Anthracnose	*Colletotrichum lagenarium*
		Leaf spot, black rot	*Didymella bryoniae*
14.	Cumin	Wilt	*Fusarium oxysporum* f. sp. *cumini*
15.	Gram	Ascochyta blight	*Ascochyta rabiei*
		Botrytis grey mould	*Botrytis cinerea*
		Fusarium wilt	*Fusarium oxysporum* f. sp. *ciceri*
16.	Jute	Leaf spot	*Cercospora corchori*
		Anthracnose	*Colletotrichum corchori*
		Stem rot, damping off	*Macrophomina phaseolina*
17.	Lentil	Blight	*Acochyta fabae* f. sp. *lentis*
		Wilt	*Fusarium oxysporum* f. sp. *lentis*
18.	Lettuce	Anthracnose	*Colletotrichum trifolii*
		Black stem	*Phoma medicaginis*
		Wilt	*Verticillium albatrum*

(continued)

Table 1.2 (continued)

S. No.	Crop	Disease	Causal organism
19.	Linseed	Blight	*Alternaria linicola*
		Grey mould	*Botrytis cinerea*
		Anthracnose, seedling blight	*Colletotrichum linicola*
		Wilt	*Fusarium oxysporum* f. sp. *lini*
		Rust	*Melampsora lini*
		Blotch	*Septoria linicola*
		Foot rot	*Phoma exigua* var. *linicola*
		Wilt	*Sclerotinia sclerotiorum*
20.	Lucerne	Anthracnose	*Colletotrichum trifolii*
		Black stem	*Phoma medicaginis*
		Wilt	*Verticillium albatrum*
21.	Mung bean	Anthracnose	*Colletotrichum truncatum*
		Charcoal rot	*Macrophomina phaseolina*
22.	Muskmelon	Wilt	*Fusarium solani* f. sp. *cucurbitae*
		Anthracnose	*Glomerella lagenaria*
		Charcoal rot	*Macrophomina phaseolina*
23.	Oats	Foot rot, foot blight	*Bipolaris sorokiniana*
		Leaf blight	*Bipolaris victoriae*
		Scab	*Fusarium graminearum*
		Kernel blight, leaf blotch	*Stagonospora avenae*
		Brown foot rot, snow mould	*Microdochium nivale*
		Leaf spot	*Drechslera avenae*
		Loose smut	*Ustilago segetum* var. *avenae*
24.	Onion	Purple blotch	*Alternaria porri*
		Damping off, green mould	*Botrytis aclada*
		White rot	*Sclerotium cepivorum*
25.	Peanut	Crown rot, collar rot	*Aspergillus niger*
		Tikka disease	*Cercospora arachidicola*
		Root rot, stem rot	*Macrophomina phaseolina*
		Collar rot, wilt	*Sclerotium rolfsii*
		Rust	*Puccinia arachidis*
		Root rot	*Rhizoctonia solani*
26.	Peas	Leaf and pod spot	*Ascochyta pinodes*
		Leaf and pod spot	*Ascochyta pisi*
		Wilt	*Fusarium oxysporum* f. sp. *pisi*
		Downy mildew	*Peronospora viciae*
		Leaf and pod spot	*Phoma medicaginis* var. *pinodella*
27.	Pearl millet	Ergot	*Claviceps fusiformis*
		Downy mildew, green ear	*Sclerospora graminicola*
		Smut	*Tolyposporium penicillariae*
28.	Pigeon pea	Anthracnose	*Colletotrichum cajani*
29.	Pumpkin	Leaf spot	*Didymella bryoniae*
		Foot rot	*Fusarium solani* f. sp. *cucurbitae*

(continued)

Table 1.2 (continued)

S. No.	Crop	Disease	Causal organism
30.	Radish	Grey leaf spot	*Alternaria brassicae*
		Black leaf spot	*Alternaria brassicicola*
		Leaf spot	*Alternaria japonica*
		Anthracnose	*Colletotrichum higginsianum*
		Damping off	*Rhizoctonia solani*
31.	Rice	Leaf spot, pink kernel, stack burn	*Alternaria padwickii*
		Black sheath rot, brown spot, seedling blight	*Bipolaris oryzae*
		Black kernel	*Curvularia* spp.
		Bakanae disease, foot rot	*Fusarium moniliforme*
		Head blight, node rot, scab	*Fusarium graminearum*
		Blast, rotten neck	*Pyricularia grisea*
		Bunt	*Tilletia barclayana*
		Leaf scald	*Microdochium oryzae*
		Sheath blight	*Rhizoctonia solani*
		Sheath rot	*Sarocladium oryzae*
		False smut	*Ustilaginoidea virens*
		Udbatta	*Ephelis oryzae*
32.	Rye	Ergot	*Claviceps purpurea*
		Blind seed disease	*Gloeotinia granigena*
		Dwarf bunt	*Tilletia controversa*
		Stem bunt	*Urocystis occulta*
33.	Safflower	Leaf spot	*Alternaria carthami*
		Leaf spot	*Cercospora carthami*
		Wilt	*Fusarium oxysporum* f. sp. *carthami*
		Rust	*Puccinia carthami*
		Wilt	*Verticillium alboatrum*
		Wilt	*Verticillium dahliae*
34.	Sesame	Blight	*Alternaria sesami*
		Leaf spot	*Alternaria sesamicola*
		Charcoal rot	*Macrophomina phaseolina*
		Brown leaf spot	*Mycosphaerella sesamicola*
35.	Sorghum	Leaf spot	*Curvularia lunata*
		Red leaf, stalk rot	*Colletotrichum graminicola*
		Seed rot, stalk rot	*Fusarium moniliforme*
		Zonate leaf spot	*Gloeocercospora sorghi*
		Downy mildew	*Peronosclerospora sorghi*
		Ergot	*Sphacelia sorghi*
		Loose smut	*Sporisorium cruentum*
		Head smut	*Sphacelotheca reiliana*
		Grain smut	*Sporisorium sorghi*
		Long smut	*Tolyposporium ehrenbergii*

(continued)

Table 1.2 (continued)

S. No.	Crop	Disease	Causal organism
36.	Soybean	Purple stain	*Cercospora kikuchii*
		Pod blight	*Colletotrichum truncatum*
		Stem canker	*Diaporthe phaseolorum* var. *batatais*
		Stem canker	*Diaporthe phaseolorum* var. *caulivora*
		Pod and stem blight	*Diaporthe phaseolorum* var. *sojae*
		Charcoal rot	*Macrophomina phaseolina*
		Downy mildew	*Peronospora manshurica*
		Root and stem rot	*Phytophthora megasperma* var. *sojae*
		Aerial blight	*Rhizoctonia solani*
37.	Spinach	Wilt	*Fusarium oxysporum* f. sp. *spinaciae*
		Downy mildew	*Peronospora farinosa* f. sp. *spinaciae*
38.	Sunflower	Leaf spot	*Alternaria helianthi*
		Grey mould	*Botrytis cinerea*
		Charcoal rot	*Macrophomina phaseolina*
		Downy mildew	*Plasmopara halstedii*
		Rust	*Puccinia helianthi*
		Wilt, white rot	*Sclerotinia sclerotiorum*
39.	Sugar beet	Leaf spot	*Cercospora beticola*
		Anthracnose	*Colletotrichum dematium* f. sp. *spinaciae*
		Downy mildew	*Peronospora farinosa* f. sp. *betae*
		Black leg	*Phoma betae*
		Rust	*Uromyces betae*
40.	Tobacco	Brown spot	*Alternaria longipes*
		Anthracnose	*Colletotrichum tabacum*
		Downy mildew	*Peronospora tabacina*
41.	Tomato	Early blight	*Alternaria solani*
		Collar rot	*Rhizoctonia solani*
		Stem canker	*Didymella lycopersici*
		Wilt	*Fusarium oxysporum* f. sp. *lycopersici*
		Leaf spot	*Septoria lycopersici*
		Anthracnose	*Colletotrichum gloeosporioides*
		Late blight	*Phytophthora infestans*
		Buckeye rot	*Phytophthora nicotianae* var. *parasitica*
42.	Watermelon	Black rot	*Didymella bryoniae*
		Anthracnose	*Glomerella lagenaria*
		Wilt	*Fusarium oxysporum* f. sp. *niveum*
		Charcoal rot	*Macrophomina phaseolina*

(continued)

Table 1.2 (continued)

S. No.	Crop	Disease	Causal organism
43.	Wheat	Alternaria leaf blight	*Alternaria triticina*
		Ergot	*Claviceps purpurea*
		Foot rot, seedling blight, spot blotch	*Bipolaris sorokiniana*
		Head blight, scab	*Fusarium graminearum*
		Glume blotch, leaf spot	*Stagonospora nodorum*
		Brown foot rot, snow mould	*Microdochium nivale*
		Yellow leaf spot	*Drechslera tritici-repentis*
		Blast	*Pyricularia grisea*
		Karnal bunt	*Tilletia indica*
		Hill bunt	*Tilletia tritici*
		Hill bunt	*Tilletia laevis*
		Dwarf bunt	*Tilletia controversa*
		Loose bunt	*Ustilago segetum* var. *tritici*
		Flag smut	*Urocystis agropyri*
44.	Zinnia	Blight	*Alternaria zinniae*

Table 1.3 Important seed-borne bacterial diseases of cereals, vegetables and oilseed crops

S. No.	Crop	Disease	Causal organism
1.	Barley	Kernel blight	*Pseudomonas syringae* pv. *syringae*
		Leaf blight	*Xanthomonas translucens* pv. *translucens*
2.	Bean	Watery soft rot, wilt	*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*
		Halo blight	*Pseudomonas savastanoi* pv. *phaseolicola*
		Bacterial blight	*Xanthomonas axonopodis* pv. *phaseoli*
3.	Brassica	Black rot	*Xanthomonas campestris* pv. *campestris*
4.	Carrot	Bacterial blight	*Xanthomonas campestris* pv. *carotae*
5.	Caster bean	Bacterial leaf spot	*Xanthomonas axonopodis* pv. *ricini*
6.	Celery	Soft rot	*Erwinia carotovora*
7.	Cotton	Angular leaf spot	*Xanthomonas axonopodis* pv. *malvacearum*
8.	Cluster bean	Blight	*Xanthomonas axonopodis* pv. *cyamopsidis*
9.	Cowpea	Bacterial blight	*Xanthomonas axonopodis* pv. *vignicola*
10.	Chilli	Bacterial leaf, stem and fruit rot	*Xanthomonas vesicatoria*
11.	Cucumber	Angular leaf spot	*Pseudomonas syringae* pv. *lachrymans*
12.	Lettuce	Wilt	*Clavibacter michiganensis* subsp. *Insidiosus*
13.	Lucerne	Wilt	*Clavibacter michiganensis* subsp. *insidiosus*
14.	Mung bean	Blight	*Xanthomonas axonopodis* pv. *phaseoli*
15.	Oats	Halo blight	*Pseudomonas syringae* pv *syringae*
16.	Peas	Bacterial blight	*Pseudomonas syringae* pv. *pisi*
17.	Pumpkin	Leaf spot	*Xanthomonas cucurbitae*

(continued)

Table 1.3 (continued)

S. No.	Crop	Disease	Causal organism
18.	Radish	Bacterial spot	*Xanthomonas campestris* pv. *raphani*
19.	Rice	Bacterial stripe	*Acidovorax avenae* subsp. *avenae*
		Bacterial grain rot	*Burkholderia glumae*
		Bacterial leaf blight	*Xanthomonas oryzae* pv. *oryzae*
		Bacterial leaf streak	*Xanthomonas oryzae* pv. *oryzicola*
20.	Sesame	Angular leaf spot	*Pseudomonas syringae* pv. *sesami*
		Bacterial leaf spot	*Xanthomonas campestris* pv. *sesami*
21.	Soybean	Bacterial blight	*Pseudomonas savastanoi* pv. *glycinea*
		Bacterial pustule	*Xanthomonas axonopodis* pv. *glycines*
22.	Tobacco	Hollow stalk	*Erwinia carotovora* subsp. *carotovora*
		Wild fire	*Pseudomonas syringae* pv. *tabaci*
23.	Tomato	Bacterial canker	*Clavibacter michiganensis* subsp. *michiganensis*
		Bacterial leaf spot	*Pseudomonas syringae* pv. *tomato*
		Bacterial spot	*Xanthomonas vesicatoria*
24.	Wheat	Tundu, yellow ear rot	*Rathayibacter tritici*
		Black chaff	*Xanthomonas translucens* pv. *translucens*
25.	Zinnia	Blight	*Xanthomonas campestris* pv. *zinniae*

Table 1.4 Important seed-borne nematode diseases of cereals and vegetable seed crops

S. No.	Crop	Disease	Causal organism
1.	Lettuce	Stem nematode	*Ditylenchus dipsaci*
2.	Lucerne	Stem nematode	*Ditylenchus dipsaci*
3.	Onion	Eelworm rot	*Ditylenchus dipsaci*
4.	Rice	White tip	*Aphelenchoides besseyi*
5.	Wheat	Ear cockle	*Anguina tritici*

Table 1.5 Important seed-borne viral diseases of cereals, vegetables and oilseed crops

S. No.	Crop	Disease	Causal organism
1.	Barley	False stripe	*Barley stripe mosaic virus*
2.	Bean	Mosaic	*Bean common mosaic virus*
3.	Cowpea	Virus	*Cowpea aphid-borne mosaic virus*
		Virus	*Black eye cowpea mosaic virus*
		Virus	*Cowpea banding mosaic virus*
		Virus	*Cowpea mosaic virus*
4.	Cucumber	Green mottle	*Cucumber green mottle virus*
5.	Lentil	Mosaic	*Pea seed-borne mosaic virus*
6.	Lettuce	Mosaic	*Alfalfa mosaic virus*
		Virus	*Lucerne latent Australian virus*
7.	Lucerne	Mosaic	*Alfalfa mosaic virus*
		Virus	*Lucerne latent Australian virus*

(continued)

Table 1.5 (continued)

S. No.	Crop	Disease	Causal organism
8.	Mung bean	Mosaic	*Bean common mosaic virus*
		Leaf crinkle	*Urdbean leaf crinkle virus*
9.	Muskmelon	Necrotic spot	*Melon necrotic spot virus*
		Mosaic	*Squash mosaic virus*
10.	Peanut	Stunt	*Peanut stunt virus*
		Mottle	*Peanut mottle virus*
		Stripe	*Peanut stripe virus*
11.	Peas	Mosaic	*Pea seed-borne mosaic virus*
12.	Pumpkin	Mosaic	*Cucumber mosaic virus*
		Necrotic ring spot	*Prunus necrotic ringspot virus*
		Mosaic	*Squash mosaic virus*
13.	Soybean	Mosaic	*Soybean mosaic virus*
		Bud blight	*Tobacco ring spot virus*
14.	Tomato	Mosaic	*Tobacco mosaic virus*
15.	Watermelon	Mosaic	*Cucumber mosaic virus*
16.	Wheat	False stripe	*Barley stripe mosaic virus*

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2 Major Seed-Borne Diseases of Agricultural Crops: International Trade of Agricultural Products and Role of Quarantine

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Abstract

The global movement of agri-horti products has the potential of introducing new insect pests and pathogens which may cause potential risk to the agriculture of the importing country. The National Plant Protection Services assumes the responsibility for protecting their countries from the unwanted entry of new pathogens and for coordinating programmes to eradicate those that have recently arrived and are still sufficiently confined for their elimination to be realistic. The exclusion can be achieved by a combination of regulatory and technical approach that can ensure biosecurity for a region. In India, the Directorate of Plant Protection, Quarantine and Storage under the Ministry of Agriculture and Farmers Welfare is responsible for enforcing quarantine regulations and for quarantine inspection and disinfestation of agricultural commodities meant for commercial purpose. The imported germplasm material including transgenics are subjected to quarantine processing at the ICAR-National Bureau of Plant Genetic Resources, New Delhi. The draft of Agricultural Biosecurity Bill is under consideration by the Indian Government. The strategies for biosecurity against plant viruses include stringent quarantine measures for imported material, domestic quarantine and use of certified disease-free seeds and other planting materials within the country. Adopting a workable strategy, several pathogens of quarantine significance have been intercepted, and the risk of introduction of these pathogens in India was thus eliminated. Adopting the appropriate technique and the right strategy for pathogen detection would go a long way in ensuring safe trade/exchange of germplasm and the biosecurity of Indian agriculture from transboundary introduction of plant pathogens.

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2.1 Introduction

Plant diseases substantially reduce crop production every year, resulting in massive economic losses throughout the world. Infected seed is a primary source of infection for several destructive diseases of economically important crops and is an excellent carrier for the spread of pathogens to long distances. International trade and exchange of plant germplasm play a vital role in the long-distance spread of a devastating pathogen or its virulent pathotype/race/strain. Biosecurity encompasses all policy and regulatory frameworks (including instruments and activities) to manage risks associated with food and agriculture (including relevant environmental risks), including fisheries and forestry. It is composed of three sectors, namely, food safety, plant life and health and animal life and health. In recent years, there has been a significant growth in trade of agri-horti produce due to liberalization under WTO. The global movement of seed material is associated with the risk of introduction of new pathogens into the country of import. The present-day definition of a pest is any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products. A quarantine pest is a pest of potential economic importance to the area at risk; it may not be present there yet or present but not widely distributed and being officially controlled (https://www.ippc.int/largefiles/adopted.../en/ISPM_05_2007_En_2007-07-26.pdf).

The pathogens introduced along with international trade of seeds and other planting materials caused serious yield losses. The Irish famine of 1845, due to total failure of potato crop because of late blight pathogen (*Phytophthora infestans*) introduced from Central America, forced the people to migrate from Europe. In Sri Lanka in 1875 and by 1889, coffee rust caused by *Hemileia vastatrix* appeared, reducing coffee production by >90%. The disease entered India in 1876 from Sri Lanka, and within 10 years, the South Indian coffee industry was affected badly. Bulk import of seeds and other planting materials lacking appropriate phytosanitary measures and the indiscriminate germplasm exchange by international agencies have resulted in the spread of pathogens in new areas which were pathogen-free (Khetarpal et al. 2006). Even a low seed transmission rate of pathogen, especially viruses, may lead to an epiphytotic proportion of the disease in field, if other conditions of field spread and climate are favourable. The worldwide dissemination of several economically important viruses such as *Bean common mosaic virus* (BCMV), *Soybean mosaic virus* (SMV), *Pea seed-borne mosaic virus* (PSbMV), *Wheat streak mosaic virus* (WSMV), *Peanut mottle virus* (PMoV), etc. is ascribed to the unhindered exchange of seed lots.

A number of exotic plant pathogens were introduced in India by imported planting materials causing enormous crop losses. These included potato late blight (*Phytophthora infestans* in 1883), coffee rust (*Hemileia vastatrix* in 1879), *Banana*

bunchy top virus (BBTV) in 1940 and flag smut of wheat (*Urocystis tritici*) in 1906. These introductions emphasized the increased pace of global trade and travel that made nations vulnerable to the danger of exotic pathogens destructive to the agriculture.

The most fundamental approach to the management of a disease is to ensure that it is not present through exclusion (quarantine) or eradication. National Plant Protection Services assumes the responsibility for protecting their countries from the unwanted entry of new pests and for coordinating programmes to eradicate those that have recently arrived and are still sufficiently confined for their elimination to be realistic. The strategies for plant health management include certified disease-free vegetative propagules and seeds, chemical control, biological control, cultural control and use of resistant varieties. In case of bulk samples, working samples need to be drawn as per the norms and tested for quality control. The detection of pathogens is then carried out by the approved or available techniques. Over the years several techniques have been developed for the detection and identification of pathogens.

Detection of pathogens and diagnosis are critical for seed trade and for germplasm exchange. Early, sensitive and accurate diagnosis is indispensable for seed and vegetative planting material certification. The selection of a detection method for assessing plant health depends on the pathogen type carried in the seed and the host. The technique should be reliable for quarantine requirements; reproducible within statistical limits; economical with regard to time, labour and equipment; and rapid to provide results of large samples in the shortest time.

2.2 Diagnostics for Detection of Pathogens

The effective detection and management of pathogens in seeds and other planting materials depend on the availability of specific, sensitive, rapid, reliable and robust methods for accurate detection and identification of pathogens. A combination of conventional and modern techniques employed for the detection of pathogens is enumerated below in brief.

2.2.1 Conventional Methods

The conventional techniques which are adapted are briefly described below.

2.2.1.1 Examination of Dry seed

Examination of dry seed under a low-power stereomicroscope with magnification up to 50–60 times may reveal symptoms such as discolouration, malformation, fruiting bodies of fungi, hyphae, eggs of insects on seed surface, insect feeding holes and even bacterial spores or growth on the surface of the seed.

2.2.1.2 Examination of Seed Washing

This technique is employed for detecting various fungi, adhered to the surface of seed, and also the spore load in a short time. Weighed seeds are shaken in a known volume of water for a fixed time on a mechanical shaker. The washing is examined under a compound microscope. By the use of phase contrast microscope, unstained bacteria can also be observed. Failure to detect infection internal to the seed and inability to distinguish between spores of saprophytic fungi and spores of pathogenic fungi are the limitations of this technique.

2.2.1.3 Examination of Soaked Seed

This technique is practised for detecting paddy bunt fungus, *Neovossia horrida*. Rice seeds are soaked in 0.2% NaOH for 24 h at 25 °C. The infected seeds appear brown, dull or shiny black. The infection is confirmed by rupturing the seed in a drop of water. A stream of smut spores are released from the shiny black discolouration.

2.2.1.4 Examination of Whole Embryo

This technique is used for detecting obligate pathogens, i.e. *Ustilago segetum* var. *hordei* and *U. segetum* var. *tritici* (loose smut of barley and wheat). Seeds are soaked overnight in 10% sodium hydroxide, containing trypan blue stain at 25 °C, and washed with warm water through sieves of decreasing size, and embryos are finally cleared in lactophenol. The infected embryos under stereomicroscope reveal bluish stained mycelium which may be present in scutellum, plumule bud or the whole embryo.

2.2.1.5 Incubation Tests

Incubation tests are used effectively against surface-borne and internal infections in which the seeds are plated. The incubation period gives chance to the dormant mycelium of fungi to grow along with the host. Most regularly used incubation tests are blotter method and agar plate method, in which seeds are placed on moist blotters and agar media, respectively.

2.2.1.6 Phage Sensitivity Test

Many bacteria are sensitive to bacteriophages and as a consequence they are lysed. Formation of lytic zones around the phage spot confirms the presence of bacterium.

2.2.1.7 Staining of Inclusion Bodies

Inclusion bodies are aggregates of viral particles or special structures characteristic of virus infection or virus-induced proteins either in the nucleus or in the cytoplasm. The virus is detected by cutting sections with a razor blade and staining with Azure A and O-G. The tissue is observed under light microscope, and inclusion bodies are located and characterized.

2.2.1.8 Electron Microscopy

The transmission electron microscope (TEM) can be used directly to detect the presence of virus in the plant tissue. It reveals the shape and size of the virus particle. The shape and size of the virus particle gives an idea of the group to which it may belong. This helps in limiting the number of antisera to be used in serological tests such as ELISA for further identification of the virus, as only antisera of viruses of a particular shape can thus be used for identification (Chalam and Khetarpal 2008). However, the TEM is a very costly equipment, and its availability is limited. In addition, electron microscopy is not suited for routine virus testing; however, the highly sensitive immunosorbent electron microscopy (ISEM), developed in 1973 by Derrick, is seldom used to detect viruses or to verify results of other methods.

2.2.1.9 Growing-on Test

Some seed-borne diseases need longer duration for their expression than the normal incubation tests. The pathogens are identified based on symptoms followed by tests of infectivity/electron microscopy/ELISA in case of viruses.

2.2.1.10 Infectivity Test

Healthy young seedlings or mature plants are exposed to the infected material to produce the symptoms. This approach is quite old; however, the technique has been used successfully to detect fungi, bacteria and viruses. In case of viruses, their presence is assayed by inoculating leaf extracts of seedlings, which may or may not be showing symptoms on indicator hosts. The indicator hosts may reveal the symptoms by producing local lesions or systemic infection. Long span of time required for development of symptoms on them, requirement of large greenhouse space and the prodigious labour and time for working with large samples are the limitations of this test.

2.2.2 Serological Tests/Immunoassays

This is based on the principle that a substance having high molecular weight (>10,000 daltons), when introduced into an animal, causes the formation of specific proteins (the immunoglobulins) in the blood, which are called antibodies. The causative element is called antigen, and the blood serum containing antibodies is called antiserum. The antigen-antibody reaction can be observed in vitro as well as in situ. Serodiagnostic tests are very sensitive and reliable in detecting the presence of virus and bacteria.

Earlier serological tests based on immunoprecipitation, immunodiffusion and latex agglutination were very popular. Now-a-days enzyme-linked immunosorbent assay (ELISA) and Dot-immunobinding assay (DIBA) are the most widely used methods of serological detection of plant viruses and also bacteria as they are more sensitive than agglutination and diffusion methods, use less antibody and can be used for simultaneous handling of huge number of samples during routine testing.

2.2.2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

The adoption of ELISA test has created new interest in serological diagnosis of plant viruses. Two types of ELISA commonly used are (i) double antibody sandwich-ELISA (DAS-ELISA) and (ii) direct antigen coating-ELISA (DAC-ELISA). The advantages of ELISA are that it is reasonably sensitive; less susceptible to 'false positives'; low cost per sample; can handle large number of samples; can be subjected to automation; and detection kits are available commercially. Forty one seed-transmitted viruses which are either not known to occur in India or are known to possess virulent strains or not known to occur in India on particular host(s) have been intercepted in germplasm including transgenics imported from many countries (Chalam et al. 2005a, b, 2009a, 2014a, b, 2017; Chalam and Khetarpal 2008; Chalam 2016; Chalam and Maurya 2018).

2.2.2.2 Dot-Immunobinding Assay (DIBA)

It is a variant of ELISA test used for the detection of viruses wherein nitrocellulose membranes are used in place of microtitre plates. A few microlitres of extract of samples are blotted on the membrane which is then immersed in primary antibody (crude antiserum). The precipitated antibody is then detected with enzyme-labelled second antibody.

DIBA has an advantage over conventional ELISA as it requires only a crude specific antiserum to each of the viruses/bacteria and a single enzyme conjugate. Above all, the blotted membranes can be mailed to long distances for further processing in laboratory.

2.2.2.3 Tissue Blotting Immunoassay/Tissue Print Immunoassay/ Tissue Print Immunoblotting

Tissue blot immunoassay is a detection method similar to DIBA, except that it does not involve tissue extraction. Instead of dotted extracts, freshly cut tissue surfaces are printed directly onto nitrocellulose membranes. The antigens trapped in the tissue blots are then reacted with antibodies, conjugates and substrates in the same way as in DIBA. The method has gained popularity for many purposes, not only due to its simplicity, eliminating the need for an extraction step, but also due to its high sensitivity, comparable with or in some cases even higher than ELISA and DIBA.

2.2.2.4 Lateral Flow Strip Method

Lateral flow strip method is used for detecting viruses/bacteria and is a variation of ELISA. The antibodies are immobilized onto a test strip in specific zones. The test does not require any major equipment and kits are available. Lateral flow strips are suitable for on-site use on field with nominal training required. Sample preparation simply involves crushing the sample and mixing it with the extraction solution provided in the kit. These tests generally provide qualitative results using antibodies and colour reagents incorporated into a flow strip.

2.2.3 Molecular Methods

2.2.3.1 Polymerase Chain Reaction

This includes rapid and highly specific in vitro amplification of selected DNA sequences, for which specific primers are used. PCR method has great potential in detection of viruses/viroids/bacteria/fungi with its specificity and high sensitivity (detecting picogram quantities of pathogens). However, the necessity of having known sequences to select and synthesize suitable primers limits its application to well-characterized pathogens.

2.2.3.2 Variants of PCR

(a) **Reverse transcription-PCR (RT-PCR):** Most of the plant viruses consist of RNA, which require the introduction of a preliminary reverse transcription (RT) step before the PCR amplification process (RT-PCR), thus allowing the amplification of RNA sequences in a cDNA form. Many viruses have been detected using RT-PCR (Chalam et al. 2004, 2012a, b; Chalam and Khetarpal 2008; Siljo et al. 2014).

(b) **Immunocapture-PCR (IC-PCR):** This is a variant of PCR which utilizes antibodies to trap viral particles without prior viral RNA extraction, which would presumably facilitate its use in routine testing. Moreover, because antibodies are involved in the first step, it may be assumed that this method could also selectively detect viruses. Therefore, this method could be very useful and practical in virus indexing programme (Phan et al. 1998).

The advantages of PCR are it is highly sensitive (can detect picogram quantities of target nucleic acid); the process is automated; very rapid – the test only takes 2 hrs or less; can be used for detecting RNA or DNA; and is very useful where ELISA is not effective (viroids, geminiviruses).

(c) **Real-time PCR/real-time RT-PCR:** Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (i.e. in real time) as opposed to the endpoint detection. The real-time progress of the reaction can be viewed. Real-time PCR quantitation eliminates post-PCR processing of PCR products. This helps to increase throughput and reduce the chances of carryover contamination. No post-PCR processing (no electrophoretical separation of amplified DNA) is required.

The advantages of real-time PCR/real-time RT-PCR are: it is not influenced by non-specific amplification; amplification can be monitored real time; no post-PCR processing of products (high throughput, low contamination risk); ultrarapid cycling (30 min to 2 h); require 1000-fold less RNA than conventional assays; and is most specific, sensitive and reproducible. The technique has been successfully exploited for detecting viruses (Chalam et al. 2004, 2012a), fungi and bacteria.

2.2.3.3 Nucleic Acid Hybridization Assays

Detection of viruses/bacteria by nucleic acid hybridization is based on the specific pairing between the target nucleic acid sequence (denatured DNA or RNA) and a

complementary nucleic acid probe to form double-stranded nucleic acids. Thus, either RNA or DNA sequences may be used as probes. It has a potential of detecting trace amount of inoculum or latent infections. The method involves immobilization of a dot or spot of leaf extract on a solid matrix and the detection of viral/bacterial nucleic acid sequences in that spot using a hybridization probe.

2.2.3.4 Double-stranded RNA (dsRNA) Analysis

For unknown or poorly characterized viruses, detection methods may not be available/are not sensitive. In such cases, double-stranded RNA analysis is a quick tool that can complement the information attained from bioassays. The method includes isolation of disease-specific dsRNAs from virus-infected tissues and electrophoretic separation on a gel, which is then viewed. However, the presence of non-viral dsRNAs in healthy plants and the apparent absence of dsRNA profile in some viruses may result in false negatives or false positives. Negative dsRNA tests should be confirmed by other methods before plant material is identified as virus-free.

2.2.3.5 Microarrays – High-Throughput Technology

In the context of phytodiagnostics, the simplest analogy that could be drawn is essentially a dot-blot in reverse, where the probe rather than the sample is bound to the solid phase. The logical extension of this approach is to immobilize a number of different spatially separated probes to the solid phase such that the samples can be tested for multiple targets. DNA capture probes (or spots) for each of the genes/pathogens to be detected are immobilized onto a solid support in a spatially separated and individually addressable fashion. Nucleic acid from the sample to be tested is extracted and labelled, and this labelled nucleic acid (known as the target) is then hybridized to the array. The array is scanned such that the hybridization events can be identified and the presence of the gene/pathogen or insect pest is resolved by the pre-defined position of the DNA capture probe in the array.

Microarrays look promising for high-throughput analysis, i.e. screening of multiple viruses/bacteria/fungi simultaneously, provided the relevant sequence information is available. Microarray analysis in theory can combine detection, identification and quantification of a large number of fungi/bacteria/viruses/nematodes/insect pests in one single assay. Hundreds of tests could be run simultaneously and in a cost-effective manner (Boonham et al. 2007).

2.2.3.6 Loop-Mediated Isothermal Amplification

Loop-mediated isothermal amplification (LAMP) is a novel technique that requires only one enzyme having strand displacement activity for amplification under isothermal conditions. LAMP has a higher specificity than PCR because its four primers recognize six distinct regions on the targeted genome. LAMP has been successfully used for detection of viruses (Arif et al. 2012; Bhat et al. 2013; Siljo and Bhat 2014).

2.2.3.7 Helicase-Dependent Amplification

Helicase-dependent amplification (HDA) requires no thermocycler for enhanced isothermal DNA amplification and has been successfully used for detection of *Bean pod mottle virus* (Chalam et al. 2012a).

2.2.3.8 Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) technologies sequence the total nucleic acid content in diseased samples for the identification of pathogens by bioinformatics tools. Unlike existing methods, the metagenomics approach does not require prior knowledge of the pathogens and can potentially identify both the known and new pathogens including viruses and viroids in a disease sample (Boonham et al. 2014; Hadidi et al. 2016).

The combination of conventional and modern techniques that are used for testing seed health for different pathogens is summarized in Table 2.1.

Table 2.1 Summary of different techniques for detecting seed-borne pathogens of quarantine significance

Techniques	Fungi	Bacteria	Viruses	Viroids
Dry seed examination	+	+	+	+
Seed washing test	+	+	–	–
Soaked seed test	+	–	–	–
Whole embryo test	+	–	–	–
Incubation tests	+	+	–	–
Phage sensitivity test	–	+	–	–
Staining of inclusion bodies	–	–	+	–
Electron microscopy	–	–	+	+
Growing-on test	+	+	+	+
Infectivity test	+	+	+	+
Enzyme-linked immunosorbent assay (ELISA)	–	+	+	–
Dot-immunobinding assay (DIBA)	–	–	+	–
Tissue blotting immunoassay	–	–	+	–
Immunosorbent electron microscopy (ISEM)	–	–	+	–
Lateral flow strips	–	+	+	–
Polymerase chain reaction (PCR)	+	+	+	+
Reverse transcription-PCR (RT-PCR)	–	–	+	–
Immunocapture-RT-PCR (IC-RT-PCR)	–	–	+	–
Real-time PCR	+	+	+	+
Real-time RT-PCR	–	–	+	–
Microarrays	+	+	+	+
Loop-mediated isothermal amplification (LAMP)	+	+	+	+
Helicase-dependent amplification (HDA)	+	+	+	+
Next-generation sequencing (NGS)	+	+	+	+

2.3 Exclusion of Pathogens Through Quarantine

2.3.1 International Scenario: Imports and Exports

The international trade-related developments and the requirement of the WTO Agreements indicate that countries need to update their biosecurity or plant health services to assist pest-free import/export.

The formation of the WTO in 1995 has provided infinite opportunities for international trade of agricultural produce/products. The devastating effects resulting from diseases and insect pests introduced along with the transboundary movement of planting materials and agricultural produce and products have been reviewed. However, sanitary and phytosanitary (SPS) measures for regulating the international trade have come up. The SPS measures deal with the application of animal health, food safety and plant health regulations. It recognizes nation's rights to implement SPS measures but specifies that they must be based on science; should be applied to the extent needed to protect human, animal or plant health; and should not unreasonably discriminate between members where conditions are similar (http://www.wto.org).

The SPS Agreement targets to overcome health-related disablements of animals and plants to market access by promoting the 'establishment, recognition and application of common SPS measures by different members'. The primary reason for the use of common international standards is that these provide the necessary health protection based on scientific evidence as well as improve trade flow simultaneously.

SPS measures are defined as any measure applied within the territory of the Member State:

- To protect animal or plant life or health from risks arising from the entry, establishment or spread of pests, diseases and disease-carrying or disease-causing organisms.
- To protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-causing organisms in food, beverages or foodstuffs.
- To protect human life or health from risks arising from diseases carried by animals, plants or their products or from the entry, establishment or spread of pests.
- To prevent or limit other damage from the entry, establishment or spread of pests.

The SPS Agreement clearly denotes the three standard-setting international organizations commonly called as the 'three sisters' whose activities are relevant to its objectives: International Plant Protection Convention (IPPC) of Food and Agriculture Organization (FAO) of the United Nations, World Organization for Animal Health (OIE) and Codex Alimentarius Commission of Joint FAO/WHO. The IPPC develops the International Standards for Phytosanitary Measures (ISPMs) which provide guidelines on pest prevention, detection and eradication. To date, 43 ISPMs (https://www.ippc.int/en/core-activities/standards-setting/ispms/) have been developed (Annexure).

Governments on a voluntary basis could adopt international standards, guidelines, recommendations, etc. prior to the establishment of WTO. Although these norms shall remain voluntary, a WTO member adopting such norms is assumed to be in full compliance with the SPS Agreement.

2.3.2 National Scenario: Imports

Plant quarantine is defined as all activities designed to prevent the introduction and/or spread of quarantine pests or to ensure their official control. Quarantine pest is a pest of potential economic importance to the area at risk. It may not be present there yet or present but not widely distributed and being officially controlled (FAO 2016).

As early as 1914, the Government of India passed a comprehensive Act, known as Destructive Insects and Pests (DIP) Act, to regulate or prohibit the import of any article into India likely to carry any pest that may be destructive to any crop, or from one state to another. The DIP Act has since undergone several amendments. In October 1988, New Policy on Seed Development was announced, liberalizing the import of seeds and other planting materials. In view of this, Plants, Fruits and Seeds (Regulation of Import into India) Order (PFS Order) first promulgated in 1984 was revised in 1989. The PFS Order was further revised in the light of World Trade Organization (WTO) Agreements, and the Plant Quarantine (Regulation of Import into India) Order 2003 [hereafter referred to as PQ Order] came into force on January 1, 2004, to comply with the Sanitary and Phytosanitary Agreement (Khetarpal et al. 2006). A number of amendments of the PQ Order were notified, revising definitions and clarifying specific queries raised by quarantine authorities of various countries, with revised lists of crops under the Schedules VI and VII and quarantine weed species under Schedule VIII. The revised list under Schedules VI and VII now include 698 and 421 crops/commodities, respectively, and Schedule VIII now includes 57 quarantine weed species. The PQ Order ensures the incorporation of 'additional/special declarations' for import commodities free from quarantine pests, on the basis of pest risk analysis (PRA) following international norms, particularly for seed/planting material (http://www.agricoop.nic.in/gazette.htm). The Agricultural Biosecurity Bill was discussed in Lok Sabha in March 2013.

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture and Farmers Welfare is responsible for enforcing quarantine regulations and for quarantine inspection and disinfestation of agricultural commodities. The quarantine processing of bulk consignments of grain, pulses, etc. for consumption and seed or planting material for sowing is undertaken by the 70 plant quarantine stations located in different parts of the country (http://www.ppqs.gov.in/contactus/plant-quarantine-stations-pqs), and many pests were intercepted in imported consignments (Sushil 2016; http://plantquarantineindia.nic.in/PQISMain/Default.aspx). Import of commercial material for sowing/planting purposes is authorized only through five plant quarantine stations. The consignment being grown in isolation across the country is inspected by 42 designated inspection authorities. In addition, DPPQS has developed 22 standards on various

phytosanitary measures such as on PRA, pest-free areas for fruit flies and stone weevils, certification of facilities for treatment of wood packaging material, methyl bromide fumigation, etc. Also, two standard operating procedures have been notified on post-entry quarantine inspection and export inspection and phytosanitary certification of plants/plant products and other regulated articles (www.plantquarantineindia.org/standards.htm).

Following are seed-borne pathogens (fungi, bacteria, viruses and viroids) of quarantine significance for India which are not reported from India, included in the PQ Order 2003 as regulated pests (Chalam et al. 2005b, 2012d, 2013a, 2017; Dev et al. 2005a, b; Gupta et al. 2013; http://plantquarantineindia.nic.in/pqispub/html/PQO_amendments.htm):

Fungi

1. *Acremonium strictum* (*Acremonium* wilt)
2. *Ascochyta abelmoschi* (leaf spot)
3. *Ascochyta fabae* (leaf and pod spot)
4. *Aspergillus wentii*
5. *Beltrania* sp.
6. *Blumeriella jaapii* (cherry leaf spot)
7. *Botryosphaeria dothidea*
8. *Ceratobasidium cereale* (sharp eyespot of cereals)
9. *Cercospora abelmoschi*
10. *C. apii*
11. *C. elaeidis* (freckle)
12. *Cladosporium caryigenum*
13. *C. geniculata*
14. *Claviceps gigantea* (ergot)
15. *C. purpurea* (ergot)
16. *Colletotrichum antirrhini*
17. *C. coffeanum* var. *virulens* (coffee berry disease)
18. *C. gossypii* var. *cephalosporioides* (witches broom of cotton)
19. *C. hibisci* (anthracnose)
20. *C. higginsianum*
21. *C. linicola* (anthracnose)
22. *C. violaetricoloris* (anthracnose)
23. *Cristulariella moricola* (zonate leaf spot)
24. *Diaporthe phaseolorum* var. *caulivora* (stem canker)
25. *Dibotryon morbosum* (black knot)
26. *Didymella chrysanthemi* (ray blight)
27. *Drechslera maydis* Race T (Southern corn blight)
28. *Elsinoe phaseoli* (scab)
29. *Embellisia allii*
30. *Eutypa armeniacae* (gummosis)
31. *Fusarium culmorum* (culm rot: cereals)

32. *F. oxysporum* f. sp. *apii*
33. *F. oxysporum* f. sp. *callistephi* (wilt)
34. *F. oxysporum* f. sp. *cucumerinum* (Fusarium wilt)
35. *F. oxysporum* f. sp. *elaeidis* (vascular wilt)
36. *F. oxysporum* f. sp. *lagenariae* (bottle gourd wilt)
37. *F. oxysporum* f. sp. *matthiolae* (wilt)
38. *F. oxysporum* f. sp. *passiflorae* (base rot disease of passion fruit)
39. *F. oxysporum* f. sp. *phaseoli* (wilt of bean)
40. *F. culmorum* (culm rot: cereals)
41. *Gaeumannomyces graminis* var. *graminis* (crown sheath rot)
42. *Gloeotinia granigena* (blind seed disease: grasses)
43. *Graphium* sp.
44. *Grovensinia pyramidalis* (zonate leaf spot of Indian jujube)
45. *Heterobasidion annosum*
46. *Heteropatella antirrhini*
47. *Kabatiella caulivora* (Northern anthracnose)
48. *Kabatiella zeae* (anthracnose)
49. *Leptosphaeria maculans* (black leg)
50. *Marssonina panottoniana* (anthracnose)
51. *Marasmiellus cocophilus*
52. *Monilinia fructicola* American strain (brown rot)
53. *M. laxa* (blossom blight and fruit rot)
54. *Moniliophthora perniciosa* (witches broom disease of cacao)
55. *M. roreri*
56. *Monographella nivalis* (foot rot of cereals)
57. *Mycena citricolor* syn. *Omphalia flavida* (American leaf spot of coffee)
58. *Mycocentrospora acerina* (anthracnose of caraway, halo blight)
59. *Mycosphaerella zeae-maydis*
60. *Nectria radicicola* (black root)
61. *Nodulisporium* sp.
62. *Periconia circinata* (milo disease)
63. *Peronospora dianthi* (downy mildew)
64. *P. dianthicola* (downy mildew)
65. *P. farinosa* (downy mildew)
66. *P. hyoscyami* f. sp. *tabacina* (angular tobacco leaf spot)
67. *P. manshurica* (downy mildew)
68. *P. tabacina* (blue mould of tobacco)
69. *Pezicula alba*
70. *Phaeoacremonium aleophilum*
71. *Phaeosphaeria avenaria* f. sp. *avenaria* (leaf spot of oats)
72. *Phakopsora meibomiae* (soybean rust)
73. *Phoma andigena*
74. *P. matthiolicola* (leaf spot)
75. *Phomopsis longicolla* (*Phomopsis* seed decay, pod and stem blight)
76. *P. sclerotioides* (black spot)

77. *Phyllosticta antirrhini*
78. *Phymatotrichopsis omnivora*
79. *Physopella zeae* (tropical rust)
80. *Phytomonas staheli* (fatal wilt or heart rot)
81. *Phytophthora cryptogea* (tomato foot rot)
82. *P. katsurae* (chestnut downy mildew)
83. *P. megakarya* (black pod of cocoa)
84. *P. megasperma* var. *sojae* (root and stem rot)
85. *P. phaseoli* (downy mildew of lima bean)
86. *P. sojae* (phytophthora root and stem rot)
87. *Plasmopara halstedii* (downy mildew)
88. *Pleiochaeta setosa* (lupin leaf spot)
89. *Pleospora herbarum* (leaf blight of onion)
90. *Puccinia antirrhini*
91. *Pyrenochaeta lycopersici* (brown rot of tomato)
92. *Pyrenopeziza medicaginis* (yellow leaf blotch)
93. *Pyrenophora dictyoides* (net blotch of fescues, *Festuca* spp.)
94. *Pythium spinosum* (root rot)
95. *P. tracheiphilum* (bottom rot of lettuce)
96. *Ramularia lacteal* (white spot)
97. *Rhizopus* sp.
98. *Sclerotinia borealis* (snow blight of grass)
99. *S. homoeocarpa* (dollar spot: grasses)
100. *S. minor* (*Sclerotinia* disease of lettuce, *Sclerotinia* rot)
101. *S. trifoliorum* (*Sclerotinia* wilt)
102. *Septoria callistephi* (leaf spot)
103. *S. cucurbitarum* (*Septoria* leaf spot)
104. *S. tageticola* (leaf spot)
105. *Sorosporium spaonariae* (smut)
106. *Sphaceloma violae* (scab)
107. *S. arachidis* (scab of groundnut)
108. *Stemphylium callistephi* (leaf spot)
109. *Tilletia controversa* (dwarf bunt of wheat)
110. *Urocystis cepulae*
111. *U. violae* (smut)
112. *Uromyces dianthi* (rust)
113. *Venturia carpophila* (scab)
114. *V. cerasi* (scab)
115. *Verticillium alboatrum*

Bacteria

1. *Acidovorax avenae* subsp. *avenae* (bacterial blight)
2. *A. avenae* subsp. *citrulli* (bacterial fruit blotch of watermelon)

3. *Burkholderia andropogonis* (bacterial blight, bacterial leaf stripe of sorghum and corn)
4. *B. glumae*
5. *B. plantarii*
6. *B. solanacearum* African strains (bacterial wilt of groundnut)
7. *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker)
8. *C. michiganensis* subsp. *nebraskensis* (Nebraska wilt)
9. *C. michiganensis* subsp. *sepedonicus*
10. *Corynebacterium michiganense* pv. *insidiosum* (bacterial wilt)
11. *Curtobacterium flaccumfaciens* pv. *betae* (silvering disease)
12. *C. flaccumfaciens* pv. *flaccumfaciens* (bacterial wilt)
13. *Erwinia rhapontici* (*Pectobacterium rhapontici*) (rhubarb crown rot)
14. *Pantoea agglomerans*
15. *P. stewartii* (bacterial leaf blight of maize)
16. *P. stewartii* sub sp. *stewartii* (Stewart's wilt)
17. *Phyllosticta impatiens*
18. *Pseudomonas ananas*
19. *P. atrofaciens* (spike rot of wheat)
20. *P. cepacia*
21. *P. cichorii* (bacterial blight, leaf spot of coffee)
22. *P. fuscovaginae* (bacterial rot of rice sheaths, sheath brown rot of rice)
23. *P. glumae* (seedling rot of rice)
24. *P. marginalis* pv. *marginalis*
25. *P. passiflora*
26. *P. putida*
27. *P. savastanoi* pv. *phaseolicola* (halo blight of beans)
28. *P. syringae* pv. *aptata* (bacterial blight)
29. *P. syringae* pv. *atrofaciens* (basal: wheat glume rot)
30. *P. syringae* pv. *atropurpurea*
31. *P. syringae* pv. *coronafaciens* (chocolate spot of maize, halo blight)
32. *P. syringae* pv. *delphinii* (leaf spot)
33. *P. syringae* pv. *lachrymans* (angular leaf spot)
34. *P. syringae* pv. *maculicola* (bacterial leaf spot, cabbage leaf spot)
35. *P. syringae* pv. *persicae* syn. *P. morsprunorum* (bacterial die back of peach)
36. *P. syringae* pv. *primulae* (leaf spot)
37. *P. syringae* pv. *punctulens* (bacterial pustule)
38. *P. syringae* pv. *triafaciens*
39. *P. syringae* pv. *tabaci* (wildfire)
40. *P. syringae* pv. *tagetis* (bacterial: *Tagetes* spp. leaf spot)
41. *P. syringae* pv. *tomato* (bacterial leaf spot)
42. *P. syringae* pv. *striafacians*
43. *P. syringae* pv. *atrofaciens* (glume rot)
44. *P. syringae* pv. *garcae* (halo blight of coffee)
45. *P. viridiflava* (bacterial leaf blight of tomato)
46. *Rhizobium rhizogenes* (gall)

47. *Xanthomonas arboricola* pv. *corylina* (hazelnut blight)
48. *X. axonopodis* pv. *vitians* (leaf spot)
49. *X. campestris* pv. *campestris* (black rot)
50. *X. campestris* pv. *incanae*
51. *X. campestris* pv. *raphani* (*Raphanus* leaf spot)
52. *X. campestris* pv. *malvacearum*, African strain (bacterial blight of cotton)
53. *X. campestris* pv. *pelargonii* (bacterial spot)
54. *X. campestris* pv. *raphani* (leafspot)
55. *X. hortorum* pv. *carotae* (bacterial blight of carrot)
56. *X. melonis* (soft rot)
57. *X. translucens* pv. *translucens* (bacterial leaf streak)
58. *X. vasicola* pv. *holcicola* (bacterial leaf streak)
59. *X. vesicatoria* (bacterial scab)
60. *Xylella fastidiosa* (Pierce's disease of grapevines)

Viruses

1. *Alfalfa mosaic virus* (AMV)
2. *Arabis mosaic virus* (ArMV)
3. *Artichoke yellow ringspot virus* (AYRSV)
4. *Asparagus virus 1* (AV-1)
5. *Asparagus virus 2* (AV-2)
6. *Barley stripe mosaic virus* (BSMV)
7. *Bean mild mosaic virus* (BMMV)
8. *Bean pod mottle virus* (BPMV)
9. *Bean yellow mosaic virus* (BYMV)
10. *Blueberry leaf mottle virus* (BLMoV)
11. *Broad bean mottle virus* (BBMV)
12. *Broad bean stain virus* (BBSV)
13. *Broad bean true mosaic virus* (BBTMV)
14. *Broad bean wilt virus* (BBWV)
15. *Cacao swollen shoot virus* (CSSV)
16. *Carnation cryptic virus 1* (CCV-1)
17. *Cherry leaf roll virus* (CLRV)
18. *Cherry rasp leaf virus* (CRLV)
19. *Citrus leaf blotch virus* (CLBV)
20. *Clover yellow mosaic virus* (ClYMV)
21. *Cocoa necrosis virus* (CoNV)
22. *Coffee ringspot virus* (CoRSV)
23. *Cowpea mottle virus* (CPMoV)
24. *Cowpea severe mosaic virus* (CPSMV)
25. *Elm mottle virus* (EMoV)
26. *Grapevine Algerian latent virus* (GALV)
27. *Grapevine chrome mosaic virus* (GCMV)

28. *Grapevine fan leaf virus* (GFLV)
29. *Grapevine line pattern virus* (GLPV)
30. *Grapevine rupestris stem pitting-associated virus* (GRSPaV)
31. *High plains virus* (HPV)
32. *Lettuce mosaic virus* (LMV)
33. *Lucerne Australian latent virus* (LALV)
34. *Maize chlorotic mottle virus* (MCMV)
35. *Melon necrotic spot virus* (MNSV)
36. *Mulberry ringspot virus* (MRSV)
37. *Odontoglossum ringspot virus* (ORSV)
38. *Papaya meleira virus* (PMeV)
39. *Pea early-browning virus* (PEBV)
40. *Pea enation mosaic virus* (PEMV)
41. *Peach rosette mosaic virus* (PRMV)
42. *Peanut stripe virus* (PStV)
43. *Peanut stunt virus* (PSV)
44. *Pepino mosaic virus* (PepMV)
45. *Pepper mild mottle virus* (PMMoV)
46. *Prune dwarf virus* (PDV)
47. *Raspberry bushy dwarf virus* (RBDV)
48. *Raspberry ringspot virus* (RpRSV)
49. *Red clover mosaic virus* (RCIMV)
50. *Red clover vein mosaic virus* (RCVMV)
51. *Satsuma dwarf virus* (SDV)
52. *Sowbane mosaic virus* (SoMV)
53. *Squash mosaic virus* (SqMV)
54. *Strawberry latent ringspot virus* (SLRSV)
55. *Sunflower crinkle virus* (SuCV)
56. *Sunn-hemp mosaic virus* (SHMV)
57. *Tobacco mosaic virus* (TMV)
58. *Tobacco necrosis virus* (TNV)
59. *Tobacco rattle virus* (TRV)
60. *Tobacco ring spot virus* (TRSV)
61. *Tobacco streak virus* (TSV)
62. *Tomato aspermy virus* (TAV)
63. *Tomato black ring virus* (TBRV)
64. *Tomato bushy stunt virus* (TBSV)
65. *Tomato mosaic virus* (ToMV)
66. *Tomato ringspot virus* (ToRSV)
67. *Turnip yellow mosaic virus* (TYMV)
68. *Vicia cryptic virus* (VCV)
69. *Wheat streak mosaic virus* (WSMV)
70. *Zucchini yellow mosaic virus* (ZYMV)

Viroids

1. *Australian grapevine viroid* (AGVd)
2. *Avocado sunblotch viroid* (ASBVd)
3. *Chrysanthemum stunt viroid* (CSVd)
4. *Coconut cadang-cadang viroid* (CCCVd)
5. *Coconut tinangaja viroid* (CTiVd)
6. *Potato spindle tuber viroid* (PSTVd)

The details of some economically important pathogens of quarantine significance for India (Dev et al. 2005b; CAB International 2007; Chalam et al. 2012c, Chalam et al. 2013a, b, c, d; Singh et al. 2012a, b) are given in Table 2.2.

The ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), the nodal organization for plant genetic resource (PGR) exchange, has been empowered under the PQ Order to handle quarantine processing of germplasm including transgenics imported for research purposes by both public and private sectors. ICAR-NBPGR has well-equipped laboratories and post-entry quarantine greenhouses. Keeping in view the biosafety requirements, National Containment Facility of level-4 (CL-4) has been established at ICAR-NBPGR to ensure that no viable pollen/biological material/pathogen enters or leaves the facility during quarantine processing of transgenics. Till date, >15,000 samples of transgenic crops comprising *Arabidopsis thaliana*, *Brassica* spp., *Cicer arietinum*, *Eucalyptus* sp., *Glycine max*, *Gossypium* spp., *Manihot esculenta*, *Nicotiana tabaccum*, *Oryza sativa*, *Solanum lycopersicum*, *S. tuberosum*, *Triticum aestivum* and *Zea mays* with different traits imported into India for research purposes were quarantined and tested for exotic pests and also for confirming the absence of terminator gene technology (embryogenesis deactivator gene) as per legislative requirements. At ICAR-NBPGR, some of the important pathogens intercepted include fungi *Peronospora manshurica*, *Uromyces betae* and *Fusarium nivale* and bacterium *Xanthomonas campestris* pv. *campestris* (Dev et al. 2012; Singh et al. 2015). In the last three decades by adopting a feasible methodology, viz. PEQ growing in PEQ greenhouses/containment facility and inspection, PEQ inspection at indenter's site, electron microscopy, enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR), 43 seed-transmitted viruses of great economic and quarantine importance have been intercepted in imported germplasm including transgenics. The interceptions include 17 seed-transmitted viruses not yet reported from India, viz. *Barley stripe mosaic virus* (BSMV), *Bean mild mosaic virus* (BMMV), *Bean pod mottle virus* (BPMV), *Broad bean mottle virus* (BBMV), *Broad bean stain virus* (BBSV), *Broad bean true mosaic virus* (BBTMV), *Cherry leaf roll virus* (CLRV), *Cowpea mottle virus* (CPMoV), *Cowpea severe mosaic virus* (CPSMV), High Plains virus (HPV), *Maize chlorotic mottle virus* (MCMV), *Pea enation mosaic virus* (PEMV), *Peanut stunt virus* (PSV), *Pepino mosaic virus* (PepMV), *Raspberry ringspot virus* (RpRSV), *Tomato ringspot virus* (ToRSV) and *Wheat streak mosaic virus* (WSMV). Besides, 21 seed-transmitted viruses not known to occur on particular host(s) in India have been intercepted and these are also of

Table 2.2 Some economically important pathogens of quarantine significance for India

Scientific name and common name	Pathway of introduction	Host range	Geographical distribution	Remarks
[a,b]***Claviceps purpurea*** (Fr.) Tul. Ergot	Seed, inflorescence, soil	*Aegilops* spp., *Agropyron* sp., *Agrostis* sp., *Avena sativa*, *Bromus* sp., *Cynodon* sp., *Dactylis* sp., *Echinochloa* sp., *Festuca* sp., *Hordeum* sp., *H. vulgare*, *Lolium* sp., *L. perenne*, *Panicum* sp., *Pennisetum* sp., *Phleum* sp., *Poa* sp., *P. pratensis*, *Secale* sp., *S. cereale*, *Sorghum* sp., *Triticum* sp., *T. aestivum*	Algeria, Argentina, Armenia, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Chile, China, Colombia, Czechoslovakia (erstwhile), Denmark, Ethiopia, Finland, France, Germany, Greece, Guinea, Hungary, Iceland, India, Iran, Ireland, Israel, Italy, Japan, Kazakhstan, Kenya, Kirgizia, Korea (DPR), Korea (Republic), Malawi, Mauritania, Mauritius, Mexico, Morocco, Nepal, Netherlands, New Zealand, Norway, Peru, Philippines, Poland, Portugal, Romania, Russian Federation, South Africa, Spain, Sudan, Sweden, Switzerland, Tanzania, Turkey, UK, Uruguay, USA, Yugoslavia (erstwhile), Zimbabwe	In the USA, high ergot incidence [(5% of potential seed sites of Kentucky bluegrass (*Poa pratensis*)] caused a 20% reduction in seed yield. The presence of ergot reduces seed quality. In cereal grains, ergot infection will result in a lower grade standard if it is present above established limits. Feed quality is reduced because of toxic alkaloids produced by the fungus. Individual countries have established regulatory limits, including import restrictions and grading standards for ergot in grain, flour, or feed products (CAB International 2007)
				Though CMI Map (1973) reports *C. purpurea* from seven states in India, there is no report of its occurrence in India (Joshi et al. 1978)
				C. purpurea was intercepted on imported germplasm of various grasses (Khetarpal et al. 2006)
[a,b]***Monographella nivalis*** (Schaffnit) E. Müll.	Seed, inflorescence, soil	*Aegilops* sp., *Agrostis canina*, *A. capillaris*, *A. stolonifera*, *Avena sativa*, *Bromus* sp., *Festuca rubra*, *Hordeum vulgare*, *Lolium multiflorum*, *L. perenne*, *Oryza sativa*, *Poa annua*, *P. pratensis*, *Secale cereale*, *Triticum aestivum*	Austria, Australia, Bahrain, Belgium, Bolivia, Brazil, Canada, China, Colombia, Czech Republic, Denmark, Estonia, Ethiopia, Finland, France, Germany, Iceland, Ireland, Italy, Latvia, Lithuania, Netherlands, New Zealand, Norway, Pakistan, Poland, Romania, Russian Federation, Senegal, Switzerland, Ukraine, UK, USA, Yugoslavia (erstwhile), Zambia	Yield losses up to 40–45% reported in Ireland and UK, respectively
Foot rot of cereals, head blight of cereals, pink snow mould, seedling blight of cereals, snow blight of cereals, snow mould of cereals				Variability of strains and differential host preference exist in fungus (Simpson et al. 2000)
				Fungicide-resistant strains reported in Sweden (Olvang and Kroeker 1987; Tvaruzek et al. 2000)

(continued)

Table 2.2 (continued)

Scientific name and common name	Pathway of introduction	Host range	Geographical distribution	Remarks
[a,b]***Tilletia controversa*** J. G. Kühn Dwarf bunt of wheat, dwarf rye bunt	Seed, inflorescence, soil	*Aegilops* sp., *Agropyron* spp., *Alopecurus myosuroides*, *A. agrestis*, *Arrhenatherum elatius*, *Bromus* spp., *Dactylis glomerata*, *Elymus* spp., *Festuca* spp., *Hordeum bulbosum*, *H. vulgare*, *Koeleria macrantha*, *Lolium* spp. *Poa* spp., *Secale cereale*, Triticale, *Triticum aestivum*	Albania, Afghanistan, Algeria, Argentina, Armenia, Australia, Austria, Azerbaijan, Bulgaria, Canada, Croatia, Czech Republic, Germany, Greece, Hungary, Iran, Iraq, Italy, Japan, Kazakhstan, Libya, Luxembourg, Moldova, Morocco (unconfirmed record), New Zealand (unconfirmed record), Poland (unconfirmed record), Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Tajikistan, Tunisia, Turkey, Turkmenistan, Ukraine, Uruguay (unconfirmed record), USA, Uzbekistan, Yugoslavia (erstwhile)	In the 1970s, the disease was reported to be of great economic importance in the EPPO region in Austria, Poland and the former USSR. Yield losses up to 30% were reported in Germany Dwarf bunt is a disease of high quarantine significance. Some countries have zero tolerance for the bunt spores in imported seeds/grains due to the possibility of spread of the disease to uninfected areas
[a,b,c]***Peronospora manshurica*** (Naumov) Syd. ex Gäum. Downy mildew of soybean	Seed, fruit, plant debris	*Glycine max*, *G. soja*	Argentina, Australia, Bermuda, Bolivia, Brazil, Bulgaria, Canada, China, Croatia, Cuba, Czech Republic, Czechoslovakia (erstwhile), Denmark, Ethiopia, France, Germany, Hungary, Iran, Israel, Italy, Japan, Kazakhstan, Korea (DPR), Korea (Republic), Latvia, Mexico, Moldova, New Zealand, Philippines, Poland, Puerto Rico, Romania, Russia, Serbia and Montenegro, Siberia, Slovakia, South Africa, Sweden, Taiwan, Thailand, Turkey, Ukraine, UK, USA, Vietnam, Zimbabwe	Dunleavy (1987) reported an average yield reduction of 11.8%. Bernard (1989) reported >30 races. Race 35 was the most pathogenic, infecting 12 of the 16 differential cultivars (Marcinkowska 1991). Three more races were identified and designated as races Zhong 1, 2 and 3 (Li et al. 1992)
[a,b]***Phytophthora sojae*** Kaufm. & Gerd. [teleomorph] Phytophthora root and stem rot, root and stem rot of soybean	Seed, fruit, plant debris	*Glycine max*, *Lupinus* spp.	Argentina, Australia, Brazil, Canada, Central Russia, Chile, China, France, Iran, Italy, Japan, Korea, Republic of Russia, Pakistan, Ukraine, USA	Yield losses up to 72% were reported (Ryley et al. 1989). Fifty five physiological races had been identified on the basis of their reaction to host differentials (Abney et al. 1997; Ryley et al. 1998; Leitz et al. 2000)

Bacteria				
[a,b]***Burkholderia glumae*** (Kurita & Tabei) Urakami et al.	Seed, inflorescence, plant debris	*Oryza sativa*	China, Colombia, Japan, Korea, Philippines, Sri Lanka, Taiwan, Vietnam	Yield reduction up to 15% reported (Katana and Kawanami 2004). Physiological variations known to occur
Bacterial grain rot, bacterial seedling rot, rice seedling blight, sheath rot, grain sterility of rice, grain discoloration of rice				
[a,b]***Curtobacterium flaccumfaciens*** **pv.** ***flaccumfaciens*** (Hedges) Collins & Jones	Seed, fruit, plant debris	*Glycine max*, *Ipomoea* spp., *Lablab purpureus*, *Phaseolus coccineus*, *P. lunatus*, *P. vulgaris*, *Vigna angularis*, *V. radiata*, *V. unguiculata*, *Zornia* spp.	Australia, Brazil, Canada, Mauritius, Romania, Russia, Tunisia, USA	Sporadic yield losses up to 19% have been recorded in soybean in USA (Dunleavy 1984)
Bacterial tan spot, bean wilt				
Viruses				
[a,b,c]***Barley stripe mosaic virus*** **(BSMV)**	Seed	*Aegilops* spp., *Agropyron elongatum*, *Avena sativa*, *Bromus inermis*, *Commelina communis*, *Hordeum depressum*, *H. glaucum*, *H. vulgare*, *Triticum aestivum* (CAB International 2007; Richardson 1990)	Argentina, Australia, Brazil, Bulgaria, Canada, China, Cyprus, Czech Republic, Denmark, Egypt, France, Germany, Greece, Hungary, Israel, Japan, Jordan, Korea, Lebanon, Mexico, Moldova, New Zealand, Norway, Pakistan, Peru, Poland, Portugal, Romania, Russian Federation, Slovakia, Slovenia, South Africa, Switzerland, Syria, Tunisia, Turkey, Ukraine, UK, USA, Yugoslavia (erstwhile)	Between 1953 and 1970, the total loss in barley caused by this virus was calculated at more than US$ 30 million in USA
Barley stripe mosaic disease		Wild oats (*A. fatua*) can be infected occasionally (Chiko 1975)		Since seed transmission is necessary for the survival of BSMV in nature and this virus has no known vectors, a simple and extremely effective means of control involves the sowing of virus-free seed. Barley seed certification schemes in Montana (Carroll 1983) and North Dakota, USA, have prevented significant yield losses since their inception
				BSMV appears on the EPPO A2 quarantine list. EPPO recommends that barley seed should come from a seed crop subject to a growing-season inspection or that the seed should have been tested by a suitable procedure

(continued)

Table 2.2 (continued)

Scientific name and common name	Pathway of introduction	Host range	Geographical distribution	Remarks
[a,c]***Bean pod mottle virus*** **(BPMV)**	Seed, beetles (*Cerotoma trifurcata*), the main vector of the virus and other beetles *Colaspis flavida*, *C. late*, *Diabrotica balteata*, *D. undecimpunctata howardi*, *D. virgifera virgifera*, *Epicauta vittata*, *Epilachna varivestis*, *Popillia japonica*), soybean leaf miner (*Odontota horni*), grafts (Fulton 1985; Werner et al. 2002; CAB International 2007)	*Glycine max*, *Desmodium paniculatum*, *Lespedeza striata*, *Mucuna deeringiana*, *Phaseolus vulgaris*, *Trifolium incarnatum*, *Vigna unguiculata*	Brazil, Canada, Ecuador, Iran, Peru, USA	Seed transmission of 0.04–0.1% was reported in *G. max*
Bean pod mottle, pod mottle of bean				Causes yield reductions ranging from 3 to 52.4% and seed coat discoloration. Deleterious effects of BPMV infection are not limited to seed yield but extend to seed quality, because it predisposes soybean seeds to *Phomopsis* spp. infection, a major cause of poor seed quality in soybean. More than 90% seed samples harvested from BPMV-infected field plots in the USA showed mottling. Combined infection of BPMV and *Soybean mosaic virus* (SMV) reduces yield by 66%. Seed coat mottling and dark pigments have often been problematic for soybean farmers and industry as it reduces consumer acceptance (Ziems et al. 2001; Giesler et al. 2002)
				Two distinct subgroups of BPMV strains (subgroups I and II) as well as reassortants between the two subgroups were reported (Giesler et al. 2002)
[a,b,c]***Broad bean stain virus*** **(BBSV)**	Seed, weevils (*Apion arrogans*, *A. vorax*, *Sitona* sp., *S. crinita*, *S. limosus*, S. *lineatus*), pollen (PaDIL – Plant Biosecurity Toolbox 2011a)	*Cicer arietinum*, *Glycine max*, *Lathyrus* sp. (*L. odoratus*), *Lens culinaris* subsp. *culinaris*, *Macrotyloma axillare*, *Melilotus albus*, *Phaseolus vulgaris*, *Pisum sativum*, *Spinacia oleracea*, *Trifolium hybridum*, *T. incarnatum*, *Vicia faba*, *V. palaestina*, *V. sativa*, *V. faba*, *Vigna unguiculata* (Fidan and Yorganci 1990; Edeme and Hanson 2000; CAB International 2007; PaDIL – Plant Biosecurity Toolbox 2011a)	Africa (as a whole), Austria, Australia, China, Czech Republic, Egypt, Ethiopia, Germany, Hungary, Iran, Italy, Jordan, Lebanon, Libya, Morocco, Poland, Slovakia, Sudan, Syria, Tunisia, Turkey, UK, USSR (erstwhile) (CAB International 2007; PaDIL – Plant Biosecurity Toolbox 2011a)	Seed transmission rates of BBSV can be very high, up to 20% in *Vicia faba* (Edwardson and Christie 1991; Mali et al. 2003), 50% in *P. sativum* (Fiedorow and Szlachetka-Wawrzyniak 2002) and 27% in *L. culinaris* (Kumari et al. 1993; Al-Khalaf et al. 2002). Yield losses can also be high, for example, pre-flowering infection in some lentils led to a 77.4% seed yield loss (Mabrouk and Mansour 1998). The seeds from infected plants are small in size as compared to seeds from uninfected plants. The crinkling and necrotic spots (stains) on soybean seeds make them unsaleable. It has a number of different strains, which vary in the severity of symptoms they cause and seed transmission rates (Kumari et al. 1996)
Broad bean stain				

[a,c]***Cowpea severe mosaic virus*** **(CPSMV)** Cowpea severe mosaic disease	Seed, insects (*Acalymma vittatum*, *Cerotoma arcuata*, *C. ruficornis*, *C. trifurcata*, *C. variegata*, *Chalcodermus bimaculatus*, *Diabrotica balteata*, *D. speciosa*, *D. undecimpunctata*, *D. virgifera*, *Diphaulaca* sp., *Epilachna varivestis*), pollen	*Canavalia ensiformis*, *Crotalaria juncea*, *Glycine max*, *Phaseolus lathyroides*, *P. vulgaris*, *Psophocarpus tetragonolobus*, *Vigna sesquipedalis*, *V. radiata*, *V. unguiculata*	Brazil, Costa Rica, El Salvador, Peru, Puerto Rico, South Africa, Suriname, Trinidad, Tobago, USA, Venezuela (Jager 1979)	Incidence up to 100% and 50% reduction in fresh plant weight, number and weight of pods have been reported in *Vigna unguiculata* (Jager 1979).
[a,b,c]**High Plains virus (HPV)** High Plains virus disease	Seed	*Avena sativa*, *Hordeum vulgare*, *Secale cereale*, *Triticum aestivum*, *Zea mays*	USA	A fully susceptible maize hybrid may be killed in the seedling stage by early infection. Infection at a later stage of development can lead to yield reduction of up to 75% in dent maize and up to 100% in sweet corn where the crop will not meet quality standards
[a,b,c]***Pea early-browning virus*** **(PEBV)** Early browning disease of pea	Seed, nematodes (*Paratrichodorus pachydermus, P. teres*, *Trichodorus anemone*, *T. teres*, *T. pachydermus*, *T. primitivus*, *T. viruliferus*), pollen (CAB International 2007; PaDIL – Plant Biosecurity Toolbox 2011b)	*Callistephus chinensis*, *Capsella bursa-pastoris*, *Lupinus* sp. (*L. luteus*), *Medicago sativa*, *M. lupulina*, *Phaseolus vulgaris*, *Pisum sativum*, *Solanum nigrum*, *Trifolium incarnatum*, *T. pratense*, *Tropaeolum majus*, *Vicia faba*, *V. faba* var. *minor*, *Zinnia elegans* (CAB International 2007; PaDIL – Plant Biosecurity Toolbox 2011b)	Algeria, Belgium, Ethiopia, Italy, Libya, Morocco, Netherlands, Poland, Sweden, UK	Seed transmission up to 61% in *P. sativum* (Fiedorow 1983), 4% in *Nicotiana rustica* and 45% in *V. faba* (Mahir et al. 1992) and 25% in *L. luteus* had been reported (Richardson 1990)
				Fiedorow (1983) found that mechanical inoculation with PEBV alone reduced the seed yield of *V. faba* by 27%. Plants co-infected with PEBV and *Broad bean true mosaic virus* suffered 77% yield loss. The infected seeds were reported to be wrinkled and discoloured (Richardson 1990)
				In the Netherlands, there is zero tolerance for PEBV in plants within prebasic and basic seed crops. In case of certified seed production, a crop is rejected if there is more than one PEBV-infected plant in 100 square metres. Not more than two infected seeds per kilogramme are tolerated for prebasic and basic seed and not more than 12 infected seeds per 500 g for certified seed (Boulton 1996)

(continued)

Table 2.2 (continued)

Scientific name and common name	Pathway of introduction	Host range	Geographical distribution	Remarks
[a,b,c]***Peanut stunt virus*** **(PSV)**	Seed, aphids (*Aphis craccivora*, *A. spiraecola*, *Myzus persicae*), dodder	*Apium graveolens*, *Arachis hypogaea*, *Coronilla varia*, *Datura stramonium*, *Glycine max*, *Lupinus albus*, *L. luteus*, *Medicago sativa*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicon*, *Tephrosia* sp., *Trifolium hybridum*, *T. incarnatum*, *T. pratense*, *T. repens*, *T. subterraneum*, *T. vesiculosum*, *Vicia faba*, *Vigna angularis*, *V. unguiculata*	China, France, Georgia, Hungary, Italy, Japan, Korea, (DPR), Korea (Republic), Morocco, Poland, Spain, Sudan, USA	Seed transmissions of 13–18% in *Glycine max* (Lizuka and Yoshida 1988) and 0.1% in *Arachis hypogaea* (Kumar et al. 1994) have been reported
Groundnut stunt, peanut stunt				Two major strains, viz. Eastern strain and Western strain, have been reported which differ in host range, serological relationships and particle stability
[a,b,c]***Tomato ringspot virus*** **(ToRSV)**	Seed, nematodes (*Xiphinema americanum*, *X. californicum*, *X. rivesi*), grafts, pollen	*Capsicum* spp., *Cerasus vulgaris*, *Cornus florida*, *Gladiolus* sp., *Glycine max*, *Fragaria chiloensis*, *Hydrangea* sp., *Lotus corniculatus*, *Lycopersicon esculentum*, *Malus domestica*, *Nicotiana tabacum*, *Pelargonium* sp., *Prunus armeniaca*, *P. avium*, *P. domestica, P. persica*, *Ribes* sp., *Rubus idaeus*, *R. hedycarpus* subsp. procerus, *Sambucus* sp., *Taraxacum officinale*, *Trifolium pratense*, *Vaccinium corymbosum*, *Vitis vinifera*	Argentina, Australia, Belarus, Canada, Chile, China, Croatia, Cyprus, Egypt, Finland, France, Germany, Hungary, Iran, Italy, Japan, Jordan, Korea (Republic), Lithuania, Mexico, New Zealand, Oman, Pakistan, Peru, Puerto Rico, Serbia and Montenegro, Slovakia, Slovenia, Tunisia, Turkey, UK, USA, Venezuela, Yugoslavia (erstwhile)	Varying degrees of seed transmission have been reported in soybean (76%), geranium (6.9%), red clover (7%), strawberry, raspberry, pelargonium and dandelion (Scarborough and Smith 1975; Stace-Smith 1984, 1987)
Apple union necrosis, chlorosis of pelargonium, grapevine yellow vein, peach yellow bud mosaic, *Prunus* stem pitting, redcurrant chlorosis mosaic, ringspot of tomato, yellow blotch curl of raspberry, yellow bud mosaic of peach, yellow vein of grapevine				Widespread in perennial plant species and causes severe decline in productivity in North America. Converse and Stace-Smith (1971) observed that fruit from infected canes weighed 21% less than normal fruit, and the yield of diseased plants was reduced by >50%
				Four strains, viz. tobacco strain, grape yellow vein strain, apple union necrosis nepovirus and euonymus chlorotic ringspot virus, have been reported (Stace-Smith 1987)

[a,b]***Avocado sun blotch viroid*** **(ASBVd)** Avocado sun blotch	Infected propagative material, grafts, cuttings, budwoods, rooted plants for propagation, seeds (CAB International 2007; Saucedo-Carabez et al. 2014)	*Persea americana*	Australia, Colombia, Costa Rica, Guatemala, Ghana, Israel, Mexico, Peru, South Africa, Spain, Syria, Turkey, Venezuela, USA (CAB International 2007; Saucedo-Carabez et al. 2014)	Saucedo-Carabez et al. (2014) reported up to 83% reduction in the total fruit weight of avocado in Mexico Seed transmission of sun blotch was demonstrated by Wallace and Drake (1953) with a level of seed transmission in fruit from sun blotch symptom-expressing trees at about 5%. However, seed from 'recovered' or symptomless carrier trees that never express symptoms display a marked increase in seed transmission of ASBVd up to 95% (Wallace and Drake 1962). One of the principal forms of sun blotch spread is via contaminated seed sources used in nursery tree production
[a,b]***Coconut cadang-cadang viroid*** **(CCCVd)** Cadang-cadang disease, yellow mottling disease	Seed nuts, seedlings, pollen, tissue cultures	*Areca catechu*, *Arenga pinnata*, *Borassus*, *Chloris*, *Cocos nucifera*, *Corypha elata*, *Elaeis guineensis*, *Phoenix dactylifera*	Guam, Philippines, Solomon Islands	Since cadang-cadang was first recorded, it has killed more than 30 million coconut palms amounting to a loss of US$ 2400–3000 million. Annual yield loss of about 22,000 t of copra has been attributed to this disease in the Philippines (Zelazny et al. 1982). Coconut is both a vital subsistence and major cash crop in developing countries including India; hence CCCVd must be considered a serious economic threat (Hanold and Randles 1991) Seed-transmitted at a low rate can be 1 in 300 (Pacumbaba et al. 1994) Import of coconut into India is prohibited from Africa (Cameroon, Ghana, Nigeria, Togo and Tanzania), North America (Florida in USA, Mexico), Central America and Caribbean (Cayman Islands, Bahamas, Cuba, Dominican Republic, Haiti, Jamaica), the Philippines and Guam, Brazil (Atlantic Coast), Trinidad, Tobago, Grenada, St. Vincent, Barbados, Belize, Honduras, Costa Rica, El Salvador, Panama, Columbia, Venezuela and Ecuador, Surinam (Dutch Guyana), Sri Lanka as per Schedule IV of Plant Quarantine (Regulation of Import into India) Order 2003 Technical guidelines for the safe movement of coconut germplasm from CCCVd-affected areas for research purposes have been developed by Bioversity International (Frison and Putter 1993)

(continued)

Table 2.2 (continued)

Scientific name and common name	Pathway of introduction	Host range	Geographical distribution	Remarks
[a,b]***Coconut tinangaja viroid*** **(CTiVd)**	Seed nuts, seedlings, tissue cultures, cutting and pruning tools can become contaminated and spread the viroid	*Cocos nucifera*	Guam	CTiVd is similar to *Coconut cadang-cadang viroid* (CCCVd), but with that it has only 64% homology. Unlike CCCVd, it is only known to occur in one molecular form and its dimer, whereas CCCVd has four molecular forms. It is possible to detect both viroids with a general probe or primer and also to detect each selectively with specific probes or primers (Hodgson et al. 1998)
Yellow mottle decline				CTiVd causes a great deal of damage in coconut plantations in Guam, where coconut is a dominant component of the island's flora. For the landscape industry, it adds to the cost of transplanting, establishing or maintaining coconut trees. For the grower and homeowner, CTiVd reduces the number of productive coconut trees and makes fresh coconuts scarcer
				Other tropical regions including India are at risk proportional to the movement of coconut parts and propagules originating from Guam
				Import of coconut into India is prohibited from Africa (Cameroon, Ghana, Nigeria, Togo and Tanzania), North America (Florida in USA, Mexico), Central America and Caribbean (Cayman Islands, Bahamas, Cuba, Dominican Republic, Haiti, Jamaica), the Philippines and Guam, Brazil (Atlantic Coast), Trinidad, Tobago, Grenada, St. Vincent, Barbados, Belize, Honduras, Costa Rica, El Salvador, Panama, Columbia, Venezuela and Ecuador, Surinam (Dutch Guyana), Sri Lanka as per Schedule IV of Plant Quarantine (Regulation of Import into India) Order 2003
				Technical guidelines for the safe movement of coconut germplasm from CTiVd-affected areas for research, but not for commercial purposes, have been developed by Bioversity International (Frison and Putter 1993)

Source: Dev et al. (2005b); Chalam et al. (2012c, 2013a, b, c, d); Singh et al. (2012a, b)
[a]Pathogen not reported from India
[b]Pathogen included in the Plant Quarantine (Regulation of Import into India) Order 2003 (PQ Order)
[c]Pathogen intercepted during quarantine processing

quarantine significance for India. Twenty viruses have been intercepted in germplasm imported from CGIAR centres (Prasada Rao et al. 1990, 2004, 2012; Kumar et al. 1991; Khetarpal et al. 1992, 1994, 2001; Parakh et al. 1994, 2005, 2006, 2008; Singh et al. 2003; Chalam et al. 2005a, 2008, 2009b, 2012a, b, d, 2013b, c, d, 2014a, b, c, 2015a, b, 2016, 2017, 2007; Chalam and Khetarpal 2008; Chalam 2014; 2016; Chalam and Maurya 2018). Although some intercepted viruses are not known to occur in India, their potential vectors are present and so also the favourable conditions to multiply and spread the destructive exotic viruses/strains. The risk of introduction of 45 seed-transmitted viruses or their strains into India was thus excluded. All the plants infected by the viruses were uprooted and destroyed.

The infected samples were salvaged by using appropriate techniques (Singh and Khetarpal 2005), and the disease-free germplasm was only used for further distribution and conservation. If not intercepted, some of these quarantine pests could have entered in our agricultural fields and caused destruction. Thus, apart from eliminating the introduction of exotic pathogens, the harvest obtained from disease-free plants guaranteed conservation of pest-free exotic germplasm in the National Genebank.

2.3.3 National Scenario: Exports

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture and Farmers Welfare is responsible for enforcing quarantine regulations and for quarantine inspection and disinfestation of agri-horticultural commodities. All the material meant for export should be accompanied by phytosanitary certificate giving the details of the material and treatment in the model certificate prescribed under the IPPC of FAO. The Ministry of Agriculture, Government of India, has notified 161 officials to grant phytosanitary certificate for export of plants and plant materials.

The ICAR-NBPGR, the nodal organization for exchange of PGR, is vested with the authority to issue phytosanitary certificate for seed material and vegetative propagules of germplasm meant for export for research purposes after getting approval from DARE. ICAR-NBPGR undertakes detailed examination of germplasm meant for export for the presence of various pests using general and pest-specific detection techniques and issues phytosanitary certificate giving the details of the material and treatment in the model certificate prescribed under the IPPC (Chalam and Mandal 2013).

2.3.4 National Domestic Quarantine

Domestic quarantine or internal quarantine is directed to prevent the spread of introduced exotic pest or an indigenous key pest to pest-free areas within the country, and this is as per the provisions of DIP Act, 1914, and is enforced by the notification issued by the Central and State Governments. More than 30 pests have been

introduced into India, but notifications were issued against 9 only of such pests, viz. fluted scale, San Jose scale, codling moth, coffee berry borer, potato wart disease, potato cyst nematode, apple scab, BBTV and *banana mosaic virus* (Khetarpal et al. 2006). As per the notifications of the DIP Act, an introduced pest, for example, BBTV, has been declared a pest in the states of Assam, Kerala, Orissa, Tamil Nadu (TN) and West Bengal (WB); and banana, which is produced from these states, has to be given a health certificate from the state pathologist or other competent authorities to certify that it is disease-free. However, due to absence of domestic quarantine, BBTV has spread to most banana-growing areas in the country. The constraints of domestic quarantine comprise lack of pest distribution maps for most of the key pests; lack of rapid diagnostic tools/kits for quick detection/identification of exotic pests at the field level; lack of pest detection surveys for identifying the affected areas and immediate launching of eradication campaigns; absence of concerted action and enforcement of internal quarantine regulations by state governments; lack of interstate border quarantine check-posts at rail and road lines greatly favoured the free movement of planting material across the states; lack of close cooperation and effective coordination between state governments and centre for timely notification of introduced pests, lack of public awareness; lack of rigorous seed/stock certification or nursery inspection programmes to make available the pest-free seed/planting material for farmers (Bhalla et al. 2014).

There is a great need to revisit the *existing domestic quarantine* scenario for strengthening interstate quarantine check-posts and ultimately for monitoring movement of economically important pathogens. Also, there is a need to review and update the existing list of pests to be regulated under domestic quarantine. For example, BBTV and banana mosaic virus (*Cucumber mosaic virus*) need to be deleted as regulated pests under domestic quarantine as they are present across the country.

The following viruses are reported only in certain parts of the country:

- *Indian citrus ring spot virus*: Reported from Haryana, Maharashtra (MH), Punjab and Rajasthan
- *Citrus mosaic virus*: Known to be present in Andhra Pradesh (AP), Karnataka and parts of TN
- *Tomato spotted wilt virus*: Reported from TN on chrysanthemum
- *Banana bract mosaic virus*: Known to be prevalent in AP, Karnataka, Kerala and TN
- *Arabis mosaic virus*: Known to be present in AP, Karnataka and parts of TN
- *Red clover vein mosaic virus*: Known to occur on the rise in Palampur, Himachal Pradesh (HP)

Thus, there is a need to consider the above viruses and others for inclusion as regulated pests for domestic quarantine to prevent their spread to other parts of the country. Also, there is a need to effectively implement domestic quarantine. India must develop organized services of plant quarantine at state level in line with Australia and the USA.

2.3.5 The Agricultural Biosecurity Bill 2013

In order to meet the challenges of globalization and free trade, the Agricultural Biosecurity Bill 2013, was introduced in the Parliament of India on March 11, 2013. The main provisions of the Bill are to set up an autonomous authority encompassing the four sectors of agricultural biosecurity, viz. plant health, animal health, living aquatic resources (fisheries, etc.) and agriculturally important micro-organisms. It provides for modernizing the legal framework to regulate the safe movement of plants and animals within the country and in international trade, and harmonize the legal requirements of the various sectors of agricultural biosecurity. The proposed legislation is expected to ensure agricultural biosecurity of the country for common benefit and for safeguarding the agricultural economy. The Bill revokes DIP Act, 1914, and the Livestock Importation Act, 1898, and will give direct powers to the quarantine officers to destroy or deport or confiscate the consignment or lodge complaints under the Indian Penal Code.

The Bill establishes the Agricultural Biosecurity Authority of India (Authority) having functions such as (i) regulating the import and export of plants, animals and related products; (ii) preventing the introduction of quarantine pests from outside India; and (iii) implementing post-entry quarantine measures. The administrative and technical control of existing plant quarantine stations, Central Integrated Pest Management Centres and other laboratories under the DPPQS shall be transferred to and vested in the Authority (http://www.indiaenvironmentportal.org.in/files/file/Agricultural%20Biosecurity%20Bill.pdf).

2.4 Challenges in Diagnosis of Pathogens in Quarantine

The issues pertaining to quarantine methodology have been reviewed by Khetarpal (2004). The challenge prior to import is pest risk analysis (PRA). PRA is mandatory for import of new produces into India. The import permit will not be granted for the commodities not included under Schedules V, VI, and VII of the PQ Order. Hence, for import of new commodities in bulk for sowing/planting, the importer should apply to the Plant Protection Adviser to the Government of India for conducting PRA. In case of germplasm, import permit shall be issued by the Director, ICAR-NBPGR, after conducting PRA based on international standards (http://agricoop.nic.in/Gazette/Psss2007.pdf).

The PRA process requires information on pest scenario in both countries (import/export). Database on all pests, including information on geographical distribution, host range, yield losses etc., should be made accessible as a ready reckoner by the quarantine officials/scientists. ICAR- NBPGR has published edited books on pests of quarantine significance for cereals (Dev et al. 2005a), grain legumes (Chalam et al. 2012c), oilseeds (Gupta et al. 2013) and tropical and subtropical fruit crops (Bhalla et al. 2018). The Crop Protection Compendium of CAB International, UK, is a beneficial asset for global pest data (CAB International 2007).

The accuracy and speed of detection of pathogens become vital as we face challenges from intentional or unintentional introductions of pests. Concentrated efforts are in progress to improve detection methodologies. The received consignment size is very crucial from the point of quarantine processing. The working samples need to be drawn as per the international norms from bulk seed lots. The standard sampling procedures need to be followed stringently, and there is a need to develop/adapt protocols for group testing, instead of individual seed testing (Maury et al. 1985). Besides, germplasm samples are generally received in small quantity, and thus, it is a limiting factor for sampling. Also, a part of the seed needs to be deposited as voucher sample in the National Genebank in India apart from the pest-free part that has to be released. Hence, extreme care is needed to ensure that the results of the test would not indicate a false-positive or a false-negative sample. Removal of exotic viruses from germplasm by growing in PEQ greenhouses unavoidably causes a delay in the release of seeds as it takes one crop growing season to release the harvest only from the tested virus-free plants. Samples received after the specified sowing time would require the indenter to wait for another crop season. More attention needs to be given to non-destructive techniques wherever possible as this could reduce time (Khetarpal 2004; Chalam and Khetarpal 2008).

2.5 Conclusion

Detection and diagnosis of pathogens are critical for application of extenuation approaches, seed trade and germplasm exchange. There is a necessity for the *web-based information portal* for sharing diagnostic protocols and database on experts in quarantine. The molecular techniques for detection of pathogens in quarantine assume great significance but need to be improved for high throughput and user-friendliness. All quarantine laboratories need to have antisera for all the plant viruses which facilitate the interception of exotic viruses and their strains. There is a need to establish a *National Diagnostic Network for Plant Pests* in line with the USA and Australia. The Network should include antisera bank, database of primers, seeds of indicator hosts, national DNA bank of plant pests, lateral flow strips/dip sticks which can detect multiple pests, multiplex PCR/RT-PCR, real-time PCR/RT-PCR, loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HAD), microarray technology, DNA barcoding, etc. A *national biosecurity chip* for diagnosis of all current threats to crop plants would be the backbone for strengthening the programme on plant quarantine. The *National Diagnostic Network for Plant Pests*, if established, can be a storehouse of information on biology of pests, detection protocols and policies. Also, *regional working groups of experts for detection and identification of plant pests* thus need to be formed to share expertise and facilities, for example, in South Asia where the borders are contiguous. This would help in avoiding the introduction of pests not known in the region and also the transboundary movement of pests within the South Asian region.

Adopting the reliable conventional, serological and molecular techniques with an appropriate strategy for the detection of pathogens would go a long way in

ensuring the management through quarantine, disease-free germplasm exchange and trade. Besides preventing the introduction of exotic pathogens, the role of diagnostics especially advanced biotechnological interventions in certification of planting material of agri-horticultural crops against indigenous pathogens boosts production and trade.

Annexure

1. ISPM 1: Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade
2. ISPM 2: Framework for pest risk analysis
3. ISPM 3: Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms
4. ISPM 4: Requirements for the establishment of pest free areas
5. ISPM 5: Glossary of phytosanitary terms
6. ISPM 6: Surveillance
7. ISPM 7: Phytosanitary certification system
8. ISPM 8: Determination of pest status in an area
9. ISPM 9: Guidelines for pest eradication programmes
10. ISPM 10: Requirements for the establishment of pest free places of production and pest-free production sites
11. ISPM 11: Pest risk analysis for quarantine pests
12. ISPM 12: Phytosanitary certificates
13. ISPM 13: Guidelines for the notification of non- compliance and emergency action
14. ISPM 14: The use of integrated measure in a systems approach for pest risk management
15. ISPM 15: Regulation of wood packaging material in international trade
16. ISPM 16: Regulated non-quarantine pests: concept and application
17. ISPM 17: Pest reporting
18. ISPM 18: Guidelines for the use of irradiation as a phytosanitary measure
19. ISPM 19: Guidelines on lists of regulated pests
20. ISPM 20: Guidelines for phytosanitary import regulatory system
21. ISPM 21: Pest risk analysis for regulated non-quarantine pests
22. ISPM 22: Requirements for the establishment of areas of low pest prevalence
23. ISPM 23: Guidelines for inspection
24. ISPM 24: Guidelines for the determination and recognition of equivalence of phytosanitary measures
25. ISPM 25: Consignments in transit
26. ISPM 26: Establishment of pest-free areas for fruit flies (Tephritidae)
27. ISPM 27: Diagnostic protocols for regulated pests
28. ISPM 28: Phytosanitary treatments for regulated pests
29. ISPM 29: Recognition of pest-free areas and areas of low pest prevalence

30. ISPM 30: Revoked. Establishment of areas of low pest prevalence for fruit flies (Tephritidae)
31. ISPM 31: Methodologies for sampling of consignments
32. ISPM 32: Categorization of commodities according to their pest risk
33. ISPM 33: Pest free potato (*Solanum* spp.) micropropagative material and minitubers for international trade
34. ISPM 34: Design and operation of post-entry quarantine stations for plants
35. ISPM 35: Systems approach for pest risk management of fruit flies (Tephritidae)
36. ISPM 36: Integrated measures for plants for planting
37. ISPM 37: Determination of host status of fruit to fruit flies (Tephritidae)
38. ISPM 38. International movement of seeds
39. ISPM 39: International movement of wood
40. ISPM 40: International movement of growing media in association with plants for planting
41. ISPM 41: International movement of used vehicles, machinery and equipment
42. ISPM 42: Requirements for the use of temperature treatments as phytosanitary measures
43. ISPM 43: Requirements for the use of fumigation as a phytosanitary measure

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Part II

History of Seed Pathology

3 Contribution of Individuals and Organizations in the Development of Seed Pathology

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Abstract

Quality seed is a basic input for crop production in agriculture, and there is always high demand of quality seed in national and international seed trade. The seed health and thereby its quality is affected by various seed-borne pathogens including fungi, bacteria, viruses, nematodes, etc. and also by abiotic factors. The science of seed pathology is an integral part of seed science and technology, which deals with the study of seed-borne diseases, overall seed health status, and management of seed-borne diseases. This science has traveled a long journey of more than a century. Many important institutions have come into existence, and a number of technologies related to seed health testing, detection, and diagnosis of seed-borne microflora and management of the seed-borne pathogens have been developed. To investigate seed health, many tests were standardized by individual researchers and organizations. Paul Neergaard made great contribution in the development of seed pathology, and hence he is considered as the father of seed pathology. Three primary organizations publish standardized seed health testing methods, and these are the International Seed Testing Association (ISTA), the International Seed Health Initiative (ISHI), and the USDA's National Seed Health System (NSHS). Among them, ISTA is key institution which provides internationally agreed set of rules for seed sampling and testing, gives authority to seed testing laboratories, and provides international seed analysis certificates to facilitate seed trading nationally and internationally. Keeping in the view above facts, the major contributions of different individuals and various organizations are being discussed in this chapter.

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AOSA · ICAR · ISHI-Veg · ISTA · Seed health · Seed pathology

3.1 Introduction

The development of science of seed pathology was initiated since long back, but in the traceable history of ancient India, the importance of seed and seed treatment was mentioned by several prominent scholars at that time. Kautilya (321–296 BC) recorded that specific seed treatment with honey, ghee, hog fat, and cow dung ensures good germination not only to true seed but also of vegetative propagated material such as sugarcane setts. It is possible that this specific seed treatment could be leading to activation of microorganisms present on the seed surface or just under seed coat and then followed by their death on exposure to sun (Agarwal 2006). Varahamihira (505–587 AD), Kashyapa (800–900 AD), and Surapala (1000 AD) also emphasized on the importance of seed heath and seed treatment to ensure proper seedling emergence in India (Seth and Misra 1998; Nene 1999; Agarwal 2006). The similar concern about seed quality has also been mentioned in the Holy Bible. There is special emphasis on creation of seed, trueness to variety, and role of seed as the basis for all forthcoming life (Steiner 2000).

In modern world, the first reference to an association of plant parasitic microbes (nematodes) with seeds was in Act IV, Scene 3 of Shakespeare's play Love's Labour's Lost (1594) in the line "sowed cockle, reaped no corn." R. Remnant in England 1637 suspected that wheat smut or hill bunt is carried with seed. He mentioned seed treatment (probably sodium chloride) for wheat bunt, and this is the first case of protection (Horsfall 1945). About one and half century later of Shakespeare's play, Needham in 1743 observed nematodes in cockled wheat seeds and showed that *Anguina tritici* caused wheat cockles. The French botanist du Tillet showed, in 1755, that stinking or hill bunt of wheat was caused by a "poisonous substance" contained in the dust sticking on seed surfaces. In 1807, Prevost proved that stinking bunt was caused by a parasitic fungus, *Tilletia caries*. Since these early reports, about 3000 microorganisms and viruses have been shown to be seed-borne (Agarwal and Sinclair 1997), and the science of seed pathology has traveled a long journey of its recognition and establishment.

Seed pathology has been recognized as a separate specialization for a relatively brief time, and the term "seed pathology" was perhaps first used by Paul Neergaard and Mary Noble in the 1940s (Munkvold 2009). In the earlier phase of its development, seed pathology referred almost only to the detection of microorganisms in or on seeds, and this practice had been conducted already for more than a century.

The science of seed pathology is an integral part of seed science and technology. It deals with the study of seed-borne disease, overall seed health status, and management of seed-borne diseases. During its development period of almost more than a century, many important institutions have come into existence, and a number of

technologies related to seed health testing, detection, and diagnosis of seed-borne microflora and management of these seed-borne pathogens have been developed. To investigate seed health, many tests were standardized by various individual researchers and organizations. The main contributing individuals and organizations of high importance are being discussed in this chapter.

3.2 Contribution of Some Stalwarts in the Development of Seed Pathology

3.2.1 Friedrich Nobbe (1830–1922)

F. Nobbe born on June 20, 1830, was a German agricultural chemist, botanist, and seed researcher. He, as a professor of biological sciences, worked since 1868 at the Academy of Forestry and Farmers in Tharandt. In 1869, he established the first official seed testing station in the world at Tharandt (Germany) with its major function to test seeds for germination and purity (Agarwal and Sinclair 1997) and thus helped in establishing seed testing. Nobbe published *Handbook of Seed Science* in 1876 in which he mentioned occurrence of sclerotia and smut balls in relation with seed production and its distribution. But he did not describe any method to detect pathogens in seed samples except those visible to unaided eye (Agarwal and Sinclair 1997). He is considered first who published on seed testing methods. Nobbe spent three and a half decades at this station in Tharandt, till 1904, at the age of 74, when he retired. He died on September 15, 1922, in Tharandt, Germany.

3.2.2 Bessey (1886)

The association of plant pathogenic microorganisms with seeds was confirmed by Bessey (1886), when he published a list of fungi detected in seeds in Iowa, USA. During that time, the focus of the researchers involved in testing of seeds was solely on purity and germination capacity of the seeds. Prior to this, there was no described method to detect pathogens in seed samples except that visible to naked eyes (Agarwal 2006).

3.2.3 A.L. Smith

In the United Kingdom, A.L. Smith in 1903 published illustrated notes on fungi found on germinated seeds of different agricultural crops. She observed various germinating agricultural crops' seeds during 1897–1901 and recorded various fungi associated with these seeds. *Microdochium nivale* appeared to be first record of implicating seed-borne pathogen as a factor contributing inferior seed quality (Agarwal 2006).

3.2.4 L. Hiltner (1917)

He, while working in Germany in 1917, concluded that wheat seed infected with *M. nivale* germinated well but resulted in poor emergence under field conditions. Therefore, a laboratory test was developed to predict field emergence. Seed health testing thus became an important component of seed testing in seed testing stations throughout Europe; thus, seed health testing emerged as a recognizable discipline of seed testing. L. Hiltner in Germany found that the laboratory estimates of germination of cereals, especially rye, were unrealistically high and could not be depended upon as indicating field performance. Thus, one of the first seed health tests to appear was developed by Hiltner working in Germany in 1917 as he developed Hiltner's bricks stone method to observe symptoms on seedlings.

3.2.5 G.N. Dorogin

Dorogin in 1923 published systems for detection of seed-borne pathogens associated with crop seeds in the U.S.S.R. He also gave brief description of these organisms, and in 1924, an analysis of crop seeds for plant pathogens was made compulsory in Russia (Agarwal and Sinclair 1997).

3.2.6 N.L. Alcock

Nora Lilian Alcock, born in 1874, was a pioneer in the field of plant pathology. She was appointed as the first government plant pathologist in the Department of Agriculture and Fisheries, mainly to help horticulturists in Scotland in 1924. N.L. Alcock in 1931 published a list of seed-borne mycoflora of forage crops, ornamental crops, and vegetables in Scotland. In the same year of 1931, she addressed the botanical society of Edinburgh on the topic "Common Diseases Sometimes Seed-borne." She retired in 1937. N.L. Alcock was plant pathologist for the Board of Agriculture for Scotland from 1924 to 1957. Alcock died at the age of 97 on March 31, 1972 (Agarwal 2006).

3.2.7 W.F. Crosier

In the United States, W.F. Crosier was appointed as research associate at Division of Seed Investigation in 1927. He published a series of papers starting from 1926 on different aspects of seed pathology such as studies on seed-borne fungi, detection and identification of seed-borne parasites, seedling diseases and injuries, routine seed health testing procedures, and chemical seed treatment from the United States. He succeeded L.C. Doyer as chairman of ISTA committee of seed health in 1949 and continued as chairman until 1953 (Agarwal 2006).

3.2.8 R.H. Porter

R.H. Porter was an American seed pathologist. He worked from 1932 to 1946 at Iowa, USA. He taught seed technology and included "recognition of the importance of seed-borne organism," the use of fungicides in seed testing, and development of laboratory methods for measuring seed germination under adverse conditions. His work "Indexing farmers' seed lots for seed-borne organisms and response to seed disinfectants" is considered significant work in the field of seed pathology. He wrote a review article on recent development in seed technology. In this review, he covered various important aspects of seed, viz., rapid methods of measuring seed viability, relationship of hormones and seed inhibitors, recognition of importance of seed-borne organism in seed laboratory practice, use of seed fungicides in seed testing, application of statistical methods to seed testing, etc. (Porter 1949). Porter in 1949 published a list of seed-borne pathogens from the United States and damage caused by them (Porter 1949).

3.2.9 Dr. Johannes de Tempe

de Tempe joined the Government Seed Testing Station in Wageningen in October 1948 as second immediate from L.C. Doyer (as the successor of Dr. C. Wehlburg who had succeeded Doyer). He has published papers on considerable range of seed-borne pathogens and their detection and practical importance (de Tempe 1953, 1963). He retired at the age of 63 on November 1, 1977, as Head of Seed Health Department at the Government Seed Testing Station, Wageningen, the Netherlands. de Tempe with Paul Neergaard and Mary Noble produced the *Annotated List of Seed-Borne Diseases* in 1958, which was updated in 1968 and subsequently in 1979 and in 1990 (Vishunavat 2009).

3.2.10 Lucie Christina Doyer

Lucie Doyer was a renowned seed pathologist and was appointed as first official seed pathologist in 1919 at the first seed health testing laboratory in the world at Government Seed Testing Station, Wageningen, the Netherlands (Agarwal and Sinclair 1997). This first seed health testing laboratory was established in 1918. Her remarkable contribution is "Manual for the determination of seed-borne diseases" published by the British Mycological Society in 1938 (Doyer 1938) which later became a landmark publication in seed pathology (Vishunavat 2009). Doyer became first chairperson of ISTA Plant Disease Committee in 1928 and continued till her death in 1949. In her career, she put an extra effort on rules of seed health testing accepted by ISTA. She developed the widely accepted "Standard Blotter Method" for detection of fungi associated with seeds (Agarwal 2006).

3.2.11 Paul Neergaard

Paul Neergaard (February 19, 1907–November 13, 1987) was a Danish scientist, who is considered as the father of seed pathology. He started his early career as plant pathologist at J.E. Ohlsens, Copenhagen, in 1935. During his 15 years' work at this biggest horticultural seed exporter company, he analyzed 40,000 samples of horticultural seeds for seed-borne infections, and he focused his research on two most important and worldwide fungal genera, *Alternaria* and *Stemphylium.* This work resulted in internationally known monograph on Danish species of *Alternaria* and *Stemphylium* in 1945. This monograph is still considered as a standard reference book on these fungal genera. Paul Neergaard coined the term seed pathology with Mary Noble in 1940s (Munkvold 2009). From 1952 to 1966, he headed the Department of Government Plant Protection Services, Copenhagen, Denmark, and continued to work on his research interest, i.e., seed-borne pathogens. He was a founder member and first director of Danish Government Institute of Seed Pathology for Developing Countries (DGISP) Copenhagen, Denmark, which was established in April 1967. He worked as director of this institute from 1967 to 1982. He established the seed-borne nature of more than hundred pathogens with their respective hosts including mainly *Fusarium, Drechslera, Colletotrichum, Bipolaris, Myrothecium,* etc. in the collaboration with his colleagues at Danish Government Institute of Seed Pathology for Developing Countries (DGISP), Copenhagen. He pioneered the 2,4-D blotter method for detection of seed-borne fungi. He was the fourth chairperson of International Seed Testing Association (ISTA), and as chairman from 1954 to 1974, he initiated standardization of seed health testing methods during his tenure. He helped standardize methods/techniques for detection of seed-borne fungi through seed health testing workshops during his tenure. His classical contribution was the writing of two-volume textbook entitled *Seed Pathology* (Neergaard 1977), and this book is considered as reference and textbook that helped teaching and practice of seed pathology around the world (Agarwal and Sinclair 1997; Agarwal 2006). He published bulletins like "University Teaching of Seed Pathology" and "Seed: A Horse of Hunger or Source of Life." Neergaard played an important role in the organization of international cooperative testing of laboratory procedures for detection of pathogens on seed aiming at achieving uniformity and international standardization (Neergaard 1970; Agarwal 2006).

3.2.12 Mary Jessie McDonald Noble

Mary Noble (1911–2002) was an internationally known seed pathologist. In 1935, she received her Ph.D. under the guidance of mycologist and plant pathologist Dr. Malcolm Wilson. Her doctorate studies focused on mycological aspects of seed pathology, which became one of her abiding research interests. She was specialized in seed-borne diseases of cereals and clover crops. Her work on blind disease of perennial ryegrass (*Gloeotinia granigena*) is considered as classic work. She worked at the Seed Pathology section in the Agricultural Scientific Services, Department of Agriculture and Fisheries, for East Craigs, Edinburgh, Scotland, affectionately called as "Seed Testing Station" for about 35 years. She presented a report to the International Seed Testing Conference entitled "Remarks on the investigations of the purity of strain and freedom from disease." She addressed the British Association of advancement of science in Edinburgh in 1951 on "the future of development of seed pathology." She was a member of the International Seed Testing Association's (ISTA) Plant Pathology Committee from 1950 to 1971 and in 1958 produced the authoritative "Annotated List of Seed-Borne Diseases" with Paul Neergaard and de Tempe; this list was updated in 1968, 1979, and 1990, and it is also counted as one of the main contributions by Mary Noble. In 1982, she was elected as president of ISTA in the First International Symposium of Seed Pathology, held in Denmark. Apart from Mary's ISTA publications, including the development of the ISTA handbook of seed health testing, her scientific publications included several accounts of plant diseases. She covered wide range of topics, viz., blind seed diseases of ryegrass, stem eelworm of strawberry, various cereal diseases, blackleg, *Verticillium* wilt and coiled shoot of potatoes, farmer's lung, and wart disease of potatoes, all of which were having great importance in Scottish agriculture at the time she worked on them. She, along with Paul Neergaard, shared the credit for introducing the term "seed pathology." She played a major role in spreading the science of seed pathology in many countries through delivering many inspiring lectures on the history of development of seed pathology, future of seed pathology, etc. in different parts of the world (Vishunavat 2009).

3.2.13 Theunis Limonard

T. Limonard at Government Seed Testing Station, Wageningen, worked on seed health assays particularly on blotter test and coined an idea of "blotter test ecology" emphasizing the effect of environmental conditions in the expression of seed-borne pathogens. He published a modified blotter test for seed health in 1966 and also reported reduction in the percentage count of *Septoria nodorum* and *Drechslera* sp. in wheat and *Stemphylium radicinum* and *Alternaria dauci* in carrot due to pretreatment by sodium hypochlorite standardized to 1% available chlorine. Limonard in 1966 introduced deep freeze method, an alternative to the 2,4-D blotter for the detection of *Phoma* infection on corn salad, *Drechslera* spp., *Bipolaris* spp., *Fusarium* spp., and *S. nodorum* in wheat (Limonard 1966). The deep freeze blotter

method has been found easy, time-saving, and reliable and yields higher counts compared to the blotter method in case of number of seed-borne pathogens. However, it is not suited for seeds such as bean and pea seeds which become putrefying mass (de Tempe and Limonard 1966).

3.2.14 Kenneth F. Baker (1908–1996)

He made major contributions to understanding diseases of ornamental plants, seed pathology, soilborne plant pathogens, biological control, and history of plant pathology (Cook 2005). The incorporation of epidemiological concepts and management considerations as components of seed pathology helped to achieve momentum with the works of K. F. Baker during the 1970s (Munkvold 2009). K.F. Baker (1972) first identified and linked epidemiological studies with seed pathology. He described three environments in which events take place, the seed production field, postharvest environment (harvesting, processing, and storage), and the crop production field, all in relation to seed pathology. He also defined categories of pathogen-seed associations within the environments and indicated how these are related to control strategies. He published progressive order of list which includes discoveries of seed-borne pathogens including fungi, bacteria, nematodes, and viruses.

3.2.15 Suresh Behari Mathur

S.B. Mathur was born on January 3, 1936, in Agra, India. He is Indian born Danish seed pathologist who mainly worked and described the transmission of pathogens from seeds to plants, location of infection in tissues of seed, histopathology, and pathogenic potentials of new seed-borne pathogens and seed health testing methods. He wrote several books like *Histopathology of Seed-Borne Infections* and *Testing Seeds of Tropical Species for Seed-Borne Diseases* and manuals like "Common Laboratory Seed Health Testing Methods for Detecting Fungi," which describes the conventional testing methods that are used in detecting fungi associated with seeds pathology which is very helpful to know about seed-borne diseases of plant and also toward the management of seed-borne diseases. He established the seed pathology

centers in India for Asian countries and one in Tanzania for African countries. The main goal of these centers is to improve the healthy seed production to increase food production and also work on seed health issues (Vishunavat 2009). Mathur has increased the awareness of plant quarantine problems and also put pressure on ISTA to develop standard method for seed health testing. Mathur and Manandhar published a book *Fungi in Seed* which includes the information of fungi in seed over 27,000 seed samples of tropical and subtropical plants. S. B. Mathur along with K. Singh published a manual entitled "An illustrated manual on identification of some seed-borne *Aspergilli*, *Fusaria*, *Penicillia,* and their mycotoxins" which covers important seed-borne pathogens such as *Aspergillus, Fusarium*, and *Penicillium* and put emphasis on identification of fungi also based on fungal mycotoxins and other secondary metabolites (Singh et al. 1991).

3.2.16 Thirumalachar, MJ (1914–1999)

MJ Thirumalachar in association with BB Mundkur in 1947 studied morphology and reported mode of transmission of ragi smut. Thirumalachar (1967) reported Aureofungin in the control of seed-borne *Helminthosporium oryzae* infection and seedling blight of paddy. He and coworker reported seed coating with the spore suspension of *Streptomyces griseus* to the seed of tomato, chili, and tobacco, to be more effective in reducing the incidence of damage of damping off of seedlings caused by *Pythium* spp. as compared to perenox.

3.3 Role of Some Important Organizations in Seed Pathology

Several organizations played an utmost important role in the development of seed pathology. In the following section, we shall discuss the contributions of these organizations in the development of seed pathology.

3.3.1 International Seed Testing Association (ISTA)

The first step toward international cooperation in seed testing was taken at the First International Seed Testing Congress (ISTC) held in Hamburg (1906), the second largest city of Germany. During the third ISTC in Copenhagen in 1921, the European Seed Testing Association was founded. During forth ISTC in Cambridge in 1924, its activities were extended to all countries and ISTC was reconstituted under its present name, International Seed Testing Association. The primary purpose of ISTA is to develop, adopt, and publish standard procedures for sampling and testing seeds and to promote uniform application of these procedures for evaluation of seeds in international trade, while secondary purposes are to promote research in all areas of seed science and technology, to encourage cultivar certification, to organize and participate in conferences and training courses aimed at furthering these objectives, and to establish and maintain liaison with other organizations having common or related interests in seeds. ISTA cooperates with the Association of Official Seed Analysts (AOSA) of North America, the European Economic Community (EEC), the European Plant Protection Organization (EPPO), the Food and Agricultural Organization (FAO) of the United Nations, the International Union of Biological Sciences (IUBS), and the International Union of Forestry Research Organizations (IUFRO) (Agarwal and Sinclair 1997). It publishes procedures and techniques used in seed testing known as the ISTA Rules. The first ISTA international rules for seed testing were published in 1931 (Steiner et al. 2008).

There are 19 subject-focused Technical Committees of ISTA (including one editorial board of seed science and technology and one seed science advisory group) which are responsible for the development of new methodology for seed testing. The Technical Committees are made up of several members, many of which are active in more than one committee. Each committee is headed by a chair and vice chair. ISTA Technical Committees include Advanced Technologies Committee, Bulking and Sampling Committee, Editorial Board of Seed Science and Technology, Flower Seed Testing Committee, Forest Tree and Shrub Seed Committee, Germination Committee, GMO Committee, Moisture Committee, Nomenclature Committee, Proficiency Test Committee, Purity Committee, Rules Committee, Seed Health Committee, Seed Science Advisory Group, Statistics Committee, Seed Storage Committee, Tetrazolium Committee, Variety Committee, and Vigour Committee (ISTA 2018).

Seed Health Committee which is one of the ISTA TCOMs is responsible to develop and establish seed health testing method, and there are 30 different testing methods in ISTA Rules. Seed testing methods are required following characteristics such as (1) high specificity and sensitivity for pathogen, (2) simplicity and quick, (3) cost performance, and (4) repeatability and reproducibility.

3.3.1.1 Role of ISTA

As an authority in seed science and technology, ISTA continues its role as the developer of seed testing methods. Its major achievements and services provided to date are briefly the following:

- The ISTA International Rules for Seed Testing, guaranteeing worldwide annually updated, harmonized, uniform, seed testing methods.
- The ISTA accreditation program including accreditation standard, proficiency testing program, and auditing program guaranteeing worldwide harmonized, uniform, seed testing.
- The issuing of the ISTA international seed lot certificates by officially independent ISTA-accredited and ISTA-authorized laboratories.
- The promotion of research, training, publishing, and information in all areas of seed science and technology and cooperation with related organizations such as ISF, OECD, UPOV, and many others.

3.3.1.2 Benefits of ISTA

- ISTA provides the basis for ensuring the trade of quality seed by developing standard seed testing methods.
- ISTA provides a platform for research and cooperation between seed scientists worldwide.
- It promotes research and provides the opportunity for publishing and distributing of the technological data.
- ISTA guarantees worldwide harmonized, uniform seed testing through the accreditation, proficiency test, and auditing programs.
- ISTA provides services and professional development programs for furthering the education and experience of seed analysts around the world.
- ISTA provides an unbiased voice in the seed industry.

3.3.1.3 Membership Profile

The membership is a collaboration of seed scientists and seed analysts from universities, research centers, and governmental, private, and company seed testing laboratories around the world. ISTA values and promotes the diversity of membership, this being the basis for its independence from economic and political influence.

3.3.2 Association of Official Seed Analysts (AOSA)

AOSA was formed in 1908 in response to initial attempts by individual states to develop seed laws. This was the beginning of regulated seed commerce in the United States. Initial priorities included, as was defined in the constitution, an attempt to seek uniformity and accuracy in methods, results, and reports. It sets as its objective an effort to perfect and make publicly known, through publication, uniform rules for seed testing.

The Association of Official Seed Analysts (AOSA) is an organization of member laboratories. Members include official state, federal, and university seed laboratories across the United States and Canada. To assure a high standard of quality, many individuals within the AOSA member laboratories have acquired AOSA Certified Seed Analyst status through extensive training followed by a mandatory certification testing process.

3.3.3 International Seed Health Initiative for Vegetables (ISHI-Veg)

The International Seed Health Initiative (ISHI) involves itself in developing, evaluating and disseminating information on seed health testing methods. ISHI for vegetables, herbage, and field crops were established in 1994, 1997, and 1998, respectively. ISHI-Veg started as an inventive of the vegetable seed industry when Dutch and French seed companies started a project on monitoring seed health in 1994. The United States, Japan, and Israel joined the initiative and represent more than 75% of the world's vegetable seed supply, and the International Seed Federation (ISF) in 2000 took over the secretariat and financial administration (ISF 2016)

The major objectives of ISHI-Veg are (i) to secure the delivery of sufficiently healthy seeds on a worldwide basis, (ii) to asses and develop suitable test procedures, (iii) to establish adequate pathogen thresholds, (iv) to develop an information database for the control of the seed-borne pathogens of economic importance of vegetable crops, and (v) to seek recognition and cooperate with official national and international regulatory and accreditation authorities (Meijerink and van Bruekelen 1995; Meijerink 1997)

In ISHI-Veg, the International Technical Group (ITG) is responsible for formulating a seed health test plan. There are four ITGs within the Technical Coordination Groups (TCGs), namely, roots, bulbs, and leafy vegetables; bean, pea, and brassica; cucurbits; and tomato and pepper, that meet every 9–11 months (Aveling 2014). A nominated representative represents each country in the ITG but can consist of more than one representative per country depending on the crop/pathogen combination. Each TCG chairperson coordinates and maintains the ISHI-Veg comparative test plans developed by the ITGs. The TCG chairperson also represents the TCG in the ISF Phytosanitary Committee and the Policy Coordination Group (PCG), which consists of one member each from the participating countries. The PCG determines the annual budget of ISHI-Veg, develops policy guidelines to assist the TCG, decides which of the reference test methods developed by the TCG appear on the ISF website in the ISHI-Veg Manual, and determines the recommended sample size for each test method. The PCG chair reports to both the ISF Vegetable Crop Section and the Phytosanitary Committee at the annual ISF Congress. Through the presentation of a draft motion and its subsequent adoption by the Vegetable Section, a position taken by ISHI-Veg can become the position of ISF. The seed health testing methods were the bases for the position adopted by the vegetable seed sector in May 2006 titled *Guidelines for the use of seed health methods by the vegetable seed industry*, which was revised in 2010 (Aveling 2014).

The TCGs are composed of seed health scientists from public and private sectors and are responsible for the establishment of reliable test methods that (1) are clear and reproducible, (2) are practical and feasible for routine testing by technical staff, (3) give results that are indisputable, (4) function as generally accepted reference methods, (5) serve as legal references in court cases, (6) support the international

seed industry in improving product quality, and (7) serve as documentation for phytosanitary certification according to IPPC guidelines (ISF 2016). For a method to be accepted by ISHI, it must be described and available for public use or published in a peer-reviewed journal and must be approved by the TCG of the particular vegetable crop. The method then enters the validation process according to ISTA's Method Validation for Seed Testing (ISTA 2007) which includes a multi-laboratory comparative test of 6–8 company and public laboratories. The number of laboratories and samples are determined in the design of the test plan, and if less than the six laboratories required by ISTA are used, the method may be accepted as a "peer-validated method" and endorsed as an ISHI reference method. Data related to the validation of components of the method and the peer reviews are stored in the ISHI database (ISF 2016). Currently, ISHI-Veg has 21 methods in their *Manual of Seed Health Testing Methods* of which 11 have been adopted as ISTA Rules and 11 as NSHS standards (Munkvold 2009; ISF 2016).

3.3.4 National Seed Health System (NSHS)

The National Seed Health System (NSHS) is a program authorized by USDA-APHIS and administered by the Iowa State University Seed Science Center to accredit both private and public players to perform certain activities needed to support the issuance of federal phytosanitary certificates for the international movement of seed

Through the NSHS, new seed health testing methods are incorporated into the accreditation program to maintain the program on the cutting edge of technology. NSHS has approved several seed heath testing methods in various crops, viz., bean, peas, *Brassica*, carrot, cucurbits, celery, spinach, coriander, lettuce, corn salad, tomato, pepper, onion, maize, and soybean for different seed-borne pathogenic fungi, bacteria, and viruses (NSHS 2018)

One of the major contributions of the NSHS is that it enables nongovernment agencies mainly seed companies to carry out seed health testing, field inspections, and seed sampling necessary to obtain phytosanitary certificates. By doing this, NSHS provides major logistical benefits in eliminating delays in the process of shipping seed lots

Activities for which entities can obtain accreditation include:

- Laboratory seed health testing for possible plant pathogens in seeds.
- Phytosanitary inspection or disease inspection of plants grown to produce seed in the field, nursery, or greenhouse.
- Seed sampling required for laboratory seed health testing.
- Visual inspection of seed shipments at exporter's facility prior to the issuance of phytosanitary certificates.

3.3.5 Indian Council of Agricultural Research (ICAR)

Realizing the importance of seed, the Indian Council of Agricultural Research launched the All India Coordinated Research Project on seed, the National Seed Project in 1979. Based on the overall progress and development of the National Seed Project and growing importance of seed in modern agriculture, the Indian Council of Agricultural Research has upgraded the Project Coordinator Unit of National Seed Project to the status of the Project Directorate in X Plan named as "Directorate of Seed Research." It started functioning since December 31, 2004, from Kushmaur village in the district Maunath Bhanjan, UP. This directorate has been recently elevated to the institute status and renamed as Indian Institute of Seed Science. This institute has well-established plant pathology laboratory that involves in detection and diagnosis of seed-borne pathogen, seed pelleting, and seed health testing and management.

3.3.6 Division of Seed Science and Technology

In 1956, a seed health testing unit established at the Mycology Division of the Indian Agricultural Research Institute (IARI), New Delhi, India, started testing of seeds for their sanitary conditions under the leadership of Dr. D. Suryanarayan. The laboratory in India has played a key role in studying seed-borne diseases of tropical crops. Many Indian scientists have been trained in seed pathology at the Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. Several seed health testing laboratories are now in operation in India. Central Seed Testing Laboratory was established during 1960 at the Indian Agricultural Research Institute (IARI), New Delhi.

In India, the National Seed Corporation started its function during 1963. The Seed Act was formulated in December 29, 1966, and enacted during 1968 in India. The Seed Rule was implemented in India from October 2, 1969. An Association of Seed Pathologists of India (ASPI) has been formed in 1974 (Jha 1993). The Plant Quarantine Act was passed during the year 1976 and the Plant Quarantine Regulations was passed in 1981. Seed (Control) Order is proposed during 1983. The new seed policy was introduced in seed industry during 1988.

3.4 Conclusion

The science of seed pathology includes seed health testing, detection-diagnosis of seed-borne diseases, seed transmission of diseases of crops, epidemiology of seed-borne diseases, management of seed-borne and seed-associated diseases, and seed storage. This is relatively new branch of science but does exist since more than 100 years ago. A number of researchers spent their valuable lives in solving the problems related to seed. Paul Neergaard, Mary Noble, K.F. Baker, and SB Mathur

can be listed among the pioneer workers, who worked on diverse components of seed pathology and helped to gain momentum in developing the science of seed pathology. Likewise, several institutions and organizations came into existence time to time and contributed potentially in the establishment of seed pathology. Among these, ISTA, AOSA, ISHI, NSHS, ICAR, and IISS played an important role at international and national scenario. The protocols and procedures of seed health testing by these major role players, namely, ISTA, AOSA ISHI, and NSHS, helped in achieving uniform international standards in seed health testing and seed pathology and made the seed trade much easier.

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Ancient, Mid-Time, and Recent History of Seed Pathology

4

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Abstract

Seed is the basic unit of crop production. An optimum plant count depends on seed for their next progeny. Issues related to seed should be handled very carefully, since sowing of a poor-quality seed leads to undesirable loss in plant stand and hence crop yield. Focus on seed quality and seed health has been a point of serious consideration since time immemorial. Seed-related aspects are puzzling the researchers since long back. Well-developed science of seed pathology has been in existence for more than 100 years. Seed pathology includes studies on the mechanism of seed transmission, pathogenesis, epidemiology of seed-borne diseases, and control measures adopted against these diseases, ranging from crop management through chemical control to legislative precautions, as well as the technology of microbiological seed testing by covering the total range of seed-borne pathogens, viz., fungi, bacteria, viruses, nematodes, and physiological

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defects which may affect any member of the plant kingdom. The various aspects, viz., seed health, management of seed-borne diseases, seed treatments, and their detection and diagnosis techniques, were developed with the time. The systematic application of these techniques leads to the significant availability of healthy and disease-free quality seed. The institutional and technological developments in the science of seed pathology are being discussed in the present chapter.

4.1 Introduction

Seed is the basic unit of crop production. Seed pathology has been in existence for more than 100 years (Noble 1979), and the term was first coined in the 1940s by Mary Noble and Paul Neergaard (Neergaard 1983). Seed pathology includes studies on the mechanism of seed transmission, pathogenesis, epidemiology of seed-borne diseases, and control measures adopted against these diseases, ranging from crop management through chemical control to legislative precautions, as well as the technology of microbiological seed testing by covering the total range of seed-borne pathogens (fungi, bacteria, viruses, nematodes) and physiological defects which may affect any member of the plant kingdom. It is intimately dependent on a considerable range of basic/applied sciences and technologies (Neergaard and Mathur 1980). It is a well-proven fact that seeds harbor microorganisms which are hazardous for the seed and/or for the new plant originated from it, and this knowledge of seed-borne nature of microorganisms launched a new era in plant pathology. In India, seed pathology has attracted the attention of many scientists working in agricultural as well as traditional universities. So, in context of the above facts, an attempt has been made in the form of this chapter to present some major historical events in the arena of seed pathology taken place during ancient, middle, and modern era with a special emphasis on diagnostic or testing techniques, management practices, and institutional developments.

4.2 Ancient Era of Seed Pathology

Around 100 A.D., a Greek physician named Galen perceived a relationship between some mysterious dark objects found in wheat being used for bread and a malady that expressed itself in the form of abortions and hallucinations. He kept accurate notes which have become one of the earliest scientific records of a seed-borne disease, but the first really close look at these so-called microorganisms came more than 15 centuries after Galen, with the invention of microscope in the late 1600s (Schoen 1987).

Documentation regarding transmission of plant pathogens through seeds came relatively late in plant pathology history although seed has attracted the attention of agriculturists even in early days. In Surpala's *Vrikshayurveda* (800 A.D.), reference

of seed treatment with milk, honey, cow dung, cow urine, and ashes of various plants like sesamum has been made. Emphasis on good-quality seed has also been made in *Manusmriti* which explains that good seed on good land yields abundant produce (Jha 1993).

4.3 Middle Era of Seed Pathology

Leaving out the ancient period, the first reference to the association of plant parasitic microorganisms (nematodes) with seed was mentioned in the mid-1590s in the act IV, scene 3, of the very famous play "Love's Labour's Lost" written by William Shakespeare (Vishunavat 2009). After about four decades, the relationship of seed to disease in the crop produced has been reflected by the observations of Remnant in the year 1637. The studies of Hellwig (1699) had been given a factual basis by increasing suspicions of farmers that some disease agent was carried out by seed. In the early 1700s, an agriculture expert in Britain named Jethro Tull was puzzled by the sooty-black, fishy-smelling dust that often coated the cereal grains at harvest (Schoen 1987). He found that soaking the seed in salty water from Bristol Bay before planting it, helped to produce a new crop with better yield. Much later of Shakespeare's play, in 1743, Needham observed nematodes in cockled wheat seeds and showed that *Anguina tritici* caused ear cockles in wheat (Needham 1743). The French botanist du Tillet, also known as Great Grandfather of Phytopathology, showed in 1755 that stinking or hill bunt of wheat was caused by a "poisonous substance" present in the dust sticking on seed surface (Tillet 1755). Schulthess (1761) suggested seed dressing with copper sulfate against stinking smut or bunt of wheat. During 1807 in France, Prevost proved that stinking bunt of wheat was caused by a parasitic fungus, *Tilletia caries*, and also showed effectivity of copper sulfate in the management of this disease (Prevost 1807). Kuhn (1858) indicated seed-borne behavior of a nematode *Ditylenchus dipsaci* (Kuhn 1858). Frank (1883) demonstrated the internally seed-borne nature of the fungus *Colletotrichum lindemuthianum* in common bean (*Phaseolus vulgaris*) seeds (Frank 1883). In 1885, Ozanne used copper sulfate and common salt to treat sorghum seed against smut before sowing and obtained fairly satisfactory results (Jha 1993). Virus seed transmission was first studied by Adolf Eduard Mayer, a German agricultural chemist, who showed in 1886 that tobacco seeds from tobacco mosaic-infected plants yield germinated to give diseased seedlings (Mayer 1886). In 1892, Beach observed common bacteria from New York (USA) and proved the seed-borne nature of bacterium *Xanthomonas campestris* pv. *phaseoli* in common bean seeds (Beach 1892). Seed protection by application of chemicals was introduced by Saunders, Bedford, and Mackay in 1894. External transmission of a bacterium was first shown by Stewart in 1897 while studying *Erwinia stewartii* on maize seeds (Schuster and Coyne 1974). In the same year, Bolley was the first to use formaldehyde for smut control in wheat seeds.

4.4 Modern Era of Seed Pathology with Special Emphasis on Management Practices

The interests in the issues related to seed-borne pathogens were increased manifold from the twentieth century. The first bacterial pathogen recorded as seed-borne was *Xanthomonas stewartii* on corn (Smith 1909). Organic mercuries for seed treatment of wheat against smut were superseded by Riehm (1913) in Germany. Mercuric chloride was used in India for the first time in 1914 by Burns for steeping seed potato to prevent *Rhizoctonia* attack (Nene and Thapliyal 1979). External seed transmission of virus, i.e., TMV on tobacco and tomato, was initially studied by Allard in 1915. In 1915, Rolfs showed the internal transmission of *Xanthomonas campestris* pv. *malvacearum* in cotton seeds and its association with lint (Rolfs 1915). McClintock suggested in 1916 that *Cucumber mosaic virus* (CMV) was transmitted by seeds (McClintock 1916). Convincing evidence for seed transmission of virus was given by Stewart and Reddick in 1917, which showed that bean seeds extracted out from common mosaic virus-infected bean plants produced infected seedlings (Stewart and Reddick 1917). In the same year, Darnell-Smith (1917) introduced copper carbonate for dusting seed treatment of wheat seeds. Doolittle and Gilbert in 1919 demonstrated seed transmission of CMV in wild cucumber (*Echinocystis lobata*). Organomercurials were used for the first time in India by Hilson (1925) for the control of sorghum smut (Ravichandra 2013). Another early demonstration of internal seed infection by bacteria was given by Clayton in 1929 while studying *Xanthomonas campestris* pv. *campestris* in cauliflower (Clayton 1929). Uppal in 1929 reported the control of brown spot of rice caused by *Helminthosporium oryzae* by seed treatment with organomercurials in India (Nene and Thapliyal 1979). Control of grain smut of sorghum (caused by the fungus *Sphacelotheca sorghi*) by seed dressing with sulfur was suggested by Uppal and Desai in 1931. Seed transmission of *Xanthomonas phaseoli* causing common bean blight was reported by Orton (1931). Rama Murty (1933) successfully controlled foot rot of paddy by seed dressing with organomercurials (Jha 1993). In 1940, Cunningham and Sharvelle introduced chloranil as a practical organic seed protectant. The first report of Von Schemling and Kulka (1966) established that carboxin-treated seed effectively controlled internally seed-borne disease loose smut of barley caused by the fungus *Ustilago nuda*. The effectivity of seed treatment with carboxin for the control of loose smut in cereals was confirmed and extended by various workers in different countries. Chatrath et al. (1969) in India reported successful control of loose smut of wheat by seed dressing with carboxin.

4.5 Expansion of Publication Sector Related to Seed Pathology

In 1876, Nobbe's published *Samenkunde*, also called *Handbook of Seed Science*, mentioned the occurrence of sclerotia and smut balls regarding seed production and distribution. However, neither he nor Hartz (another author of that time) described

any method to detect pathogens in seed samples except those visible to the unaided eye (Wold 1983). The association of plant pathogens with seeds was verified by Bessey in 1886 in Iowa, when he published a list of fungi detected in seeds (Agarwal 2006). Later, A. L. Smith published illustrated notes on fungi. She recorded on germinated crop seeds during 1897–1901 in the United Kingdom (Smith 1903). In 1897, seed-testing rules were first prepared and published in North America by the USDA in a circular entitled *Rules and Apparatus for Seed Testing* as unofficial guide for seed analysts (Justice 1961).

Chen published a monograph on internal fungal parasites of agricultural seeds in 1920 (Chen 1920). Dorogin in 1923 published a detailed scheme for the detection of seed-borne pathogens associated with the seeds in different crop plants. At the 1924 ISTA Congress in Cambridge, Genter presented a paper on *The Determination of Plant Diseases Transmitted by Seed* (Agarwal and Sinclair 1997). In 1926, Klemm recommended Dorogin's methods for the detection of seed-borne pathogens in Germany. The *International Rules for Seed Testing* was first published by ISTA in 1928. These rules included a chapter on determination of sanitary conditions of seeds and how to report such test. Special attention was given to the fungi *Claviceps purpurea*, *Fusarium*, *Tilletia*, and *Ustilago segetum* on cereals; *Ascochyta pisi* on peas; *Colletotrichum lindemuthianum* on beans; and *Botrytis*, *Colletotrichum linicola*, and *Aureobasidium lini* on flax (Wold 1983). To meet the demands of new technology and new crops, the rules and procedures are reexamined and revised annually. For example, the development of seed-coating technology has led to alterations and additions to the "International Rules for Seed Testing" published by the ISTA in 1985 and the "Rules for Testing Seeds" as published by the AOSA in 1988. Contribution of L. C. Doyer, the first chairperson of the ISTA Plant Disease Committee, was published as ISTA Proceedings in 1930 which was a systematic survey of diseases and pests on weeds involving bacteria, fungi, insects, viruses, and other injurious organisms. This work of L. C. Doyer became the basis of her presentation at the 1931 Wageningen Congress. It is titled *Proposals for Recording the Sanitary Conditions of Seed on the International Rules of Seed Testing* and also called ISTA *International Rules of Seed Testing* (Steiner et al. 2008). Lists of seed-borne mycoflora and the damage caused by them were published in 1931 by Orton in the United States and by Alcock in Scotland. The outstanding publication, *Manual for the Detection of Seed-Borne Diseases*, was published in 1938 by Dr. L. C. Doyer. The manual explained the detection, methodology, identification, and detailed descriptions of some important seed microflora and improving the sanitary conditions by cleaning or treating them. This publication was the outcome of a joint cooperative work of the members of the International Seed Testing Association. In 1940, *Handbook on Seed Testing* was published by the Association of Official Seed Analysts (AOSA 1940). Porter in 1949 also published the lists of fungal parasites of seeds from the United States. A list of the fungi found on barley, oat, and wheat seeds was compiled in Canada by Machacek et al. (1951). The US Department of Agriculture published a *Manual for Testing Agricultural and Vegetable Seeds* in the year 1952 (USDA 1952). In 1954, the European Plant Protection Organization (EPPO) published a report of the first working party on seed-borne diseases entitled *Danger from seed-borne diseases*

which is a practical approach to the problem of International Safeguard. Two ISTA handbooks on seed testing methods were also published by de Tempe (1961) and de Tempe and Binnerts (1979). *An Annotated List of Seed-Borne Diseases*, an ISTA handbook on seed health testing, has been published thrice: firstly, by Noble, De Tempe, and Neergaard in 1958–1959, secondly the updated edition by Noble and Richardson in 1968, and thirdly the revised edition in 1979 and also in 1990 by Richardson, with a Supplement-1 published in 1981. In 1970, *Tetrazolium Testing Handbook for Agricultural Seeds* was published by the Association of Official Seed Analysts (AOSA 1970). Justice and Bass (1978) wrote a book entitled *Principles and Practices of Seed Storage*. Revised ISTA *International Rules for Seed Testing* was amended and approved on recommendations of technical committees in 1966 and again in 1990 (ISTA 2001). ISTA *International Rules for Seed Testing* was amended several times, the latest being in 2016.

Research papers published from the Institute of Seed Pathology, Copenhagen, on the identification of some of the common genera of fungi, such as *Curvularia* (Benoit and Mathur 1970), *Drechslera* (Chidambaram et al. 1973), *Colletotrichum* (Kulshrestha et al. 1976), and *Fusarium* (Ram Nath et al. 1983), are useful references. Neergaard in 1977 published a book *Seed Pathology* in two volumes which proved to be a landmark in seed pathology. A book on seed viability named *Viability of Seeds* was written by E. H. Roberts in 1972. An eminent publication in the form of a book *Seed Technology* was drafted by R. L. Agrawal during 1980 which deals with the seed industry in India and the development of seed programs along with general principles of seed production and maintenance, seed processing, storage and marketing, seed testing, certification, and their legislation. V. K. Agarwal and J. B. Sinclair (1988) published a two-volume book *Principles of Seed Pathology*, and in 1997, they wrote the second edition of *Principles of Seed Pathology*; this book was among the popular books on the subject covering different aspects of seed pathology. Dalbir Singh and SB Mathur (2004) wrote a book *Histopathology of Seed-Borne Infections* claimed as the first book which provides comprehensive coverage of seed infection and disease. V. K. Agarwal (2006) published a book named *Seed Health.* This book was more focused on seed health testing, covering a wide range of conventional and molecular seed health testing methods. In 1998, Sheppard and Wesseling published a guide for comparative testing of methods for the detection of seed-borne pathogens. This was the basis for the *ISTA handbook of method validation for the detection of seed-borne pathogens* published by Sheppard and Cockerell in 2000. At that time, Sheppard was the chairman of the Seed Health Committee (SHC). Based on this publication, a Method Validation Working Group was established in 2002 to develop the ISTA Method Validation Programme which came into force in 2007 and now applies to all seed quality testing (Hampton 2005, 2007). The term "Method Validation" is new in seed testing. According to Steiner et al. (2008), it was previously called "Method Standardization" or "Method Elaboration." The Association of Official Seed Analysts (AOSA) is an organization of member laboratories across the United States and Canada that establishes and publishes seed testing rules in the AOSA *Rules for Testing Seeds* (AOSA 2013).

4.6 Development of Seed Testing Institutes/Associations

Testing of seeds for germination and purity as a measure of seed quality has been a universal practice for more than a century. The first official seed testing station was established in 1869 by Nobbe in Tharandt (Germany) with its major function to test the seeds for germination and purity (Anonymous 1986). In 1876, another such laboratory was established in the United States by the Connecticut Agricultural Experiment Station (Schaad 1983). In 1884, E. Schribaux founded the Station Nationale d'Essais de Semences in France (Anonymous 1986). Today, most of the countries have one or more federal and/or state seed testing laboratories for assessing seed germination, purity, freedom from noxious weed seeds, moisture content, and general seed health (Justice 1961). Formalized procedures for testing seeds for germination and purity have been standardized by the Association of Official Seed Analysts (AOSA) formed in 1908 in the United States along with the International Seed Testing Association (ISTA) and US Federal Seed Act formed in 1939 (Roos and Wianer 1991). The first step toward international cooperation in seed testing was taken at the first International Seed Testing Congress (ISTC) in Hamburg in 1906. At the third ISTC in Copenhagen in 1921, the European Seed Testing Association was founded. During the fourth ISTC in Cambridge in 1924, activities of the association were extended to all countries, and ISTC was reconstituted under its present name, International Seed Testing Association (ISTA). The primary purpose of ISTA is to develop, adopt, and publish standard procedures for sampling and testing seeds and to promote uniform application of these procedures for evaluation of seeds in international trade. Secondary purposes are to promote research in all areas of seed science and technology, to encourage cultivar certification, to participate in conferences and training courses aimed at furthering these objectives, and to establish and maintain liaison with other organizations having common or related interests in seeds. ISTA cooperates with the Association of Official Seed Analysts (AOSA) of North America, the European Economic Community (EEC), the European Plant Protection Organization (EPPO), the Food and Agricultural Organization (FAO) of the United Nations, the International Union of Biological Sciences (IUBS), and the International Union of Forestry Research Organizations (IUFRO) (Agarwal and Sinclair 1997). It publishes procedures and techniques used for seed testing known as the ISTA Rules.

With the increasing request of pathogen-free seed, the first seed health testing laboratory was established in 1918 at the Government Seed Testing Station, Wageningen, the Netherlands, with L. C. Doyer as the first official seed pathologist (Yorinori et al. 1979). L. C. Doyer was the first chairperson of the ISTA Plant Disease Committee (PDC) for Seed Health Committee. P. Paul F. M. de Neergaard, also called the Father of Seed Pathology, was the chairman of the ISTA PDC from 1956 to 1974 (Fig. 4.1).

In 1920, Leningrad Plant Protection station was opened in Russia, and Dorogin undertook the work on seed-borne fungi of crops. In 1954, the European Plant Protection Organization (EPPO), a regional organization, was established in accordance with the International Plant Protection Convention (IPPC) of 1951, sponsored

Fig. 4.1 P. Paul F. M. de Neergaard (Anonymous 1985)

by FAO. The Institute of Seed Pathology for Developing Countries was opened by the Danish Government at Copenhagen in 1967 to train persons for seed health testing. The institute has made a significant contribution in the development of seed pathology. Federation of the International Seed Trade (FIS) created International Seed Network Initiative during June 1999.

In 1956, a seed health testing unit was established at the Mycology Division of the Indian Agricultural Research Institute (IARI), New Delhi, India; it started testing of seeds for their sanitary conditions under the leadership of Dr. D. Suryanarayan. This laboratory in India has played a key role in studying seed-borne diseases of tropical crops. Many Indian scientists have been trained in seed pathology at the Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. Several seed health testing laboratories are now in operation in India. Central Seed Testing Laboratory was established during 1960 at Indian Agricultural Research Institute (IARI), New Delhi. In India, the National Seed Corporation started its function during 1963. Seed Act was formulated on 29 December 1966 and enacted during 1968 in India. Seed rules were implemented in India from 2 October 1969. An Association of Seed Pathologists of India (ASPI) was formed in 1974 (Jha 1993). Plant Quarantine Act was passed during the year 1976, and Plant Quarantine Regulations were passed in 1981. Seed (Control) Order was proposed during 1983. The New Seed Policy was introduced in seed industry during 1988.

The Indian Government realized the importance of seed, and the Indian Council of Agricultural Research launched the All India Coordinated Research Project on seed known as the *National Seed Project* in 1979. Based on the overall progress and development of the National Seed Project and growing importance of seed in modern agriculture, the Indian Council of Agricultural Research upgraded the Project Coordinator Unit of National Seed Project to the status of the Project Directorate in Xth Plan named as *Directorate of Seed Research* (*DSR*). Directorate of Seed Research started operating since 31 December 2004 from Kushmaur village in the district of Maunath Bhanjan, UP.

4.7 Expansion of Diagnostic Techniques Related to Seed Pathology

Frederick Nobbe known as the Father of Seed Testing coined the word *triebkraft* during the year 1876, and the term was later adopted, irrespective of the disease factor, to denote that seeds could not only germinate but also grow well. The term *triebkraft* is intentionally or not a play on words, meaning both "shoot strength" and "driving force" (Heydecker 1972).

P. Paul F. M. de Neergaard established the first comparative seed health testing program to standardize techniques for the detection of seed-borne pathogens in 1957 (Mathur and Jorgensen 2002). The first specific seed health methods in the ISTA rules were introduced in 1966 (Mathur and Jorgensen 2002; Muschick 2010). Several methods have been developed to detect seed-borne microflora, and these have been reviewed by de Tempe (1961) and Neergaard (1973). These detection methods may be general or specific for individual pathogen.

According to Mathur and Jorgensen (2002), the dominating factor which influenced the development of seed health testing was the increased awareness of plant quarantine problems, and extra pressure was put on ISTA to develop standard methods for such testing. A. J. Skolko presented the first report on comparative seed health testing of oats, flax, cabbage, wheat, barley, and beet in 1956 at the 11th ISTA congress, and the decision was made that the PDC would continue with comparative testing.

4.7.1 Techniques Used for Detection of Seed-Borne Fungi

4.7.1.1 Direct Inspection of Seeds without Incubation

- ***Examination of Dry Seeds:*** The seed sample is first examined by the naked eye and then under stereoscopic binocular microscope (ergot of cereals, purple stain of soybean, black point of wheat, etc.) to record the observations on the mixture of other crop seeds, weed seeds, plant parts, inert matter, discolorations, malformations, fungal structures like encrusted mycelium, sclerotia, galls, bunt balls, acervuli, pycnidia, perithecia, spore masses, etc. (de Tempe and Binnerts 1979).
- ***Washing Test of Infested Seeds:*** This method is suitable for the detection of the most common causal agents of diseases that contaminate seeds. With the washing test, the inoculum present on the seed surface (oospores of downy mildew fungi, teliospores of smuts and bunts, etc.) is detected. This was first done by Keitreiber in 1984 for the detection of *Tilletia* spp. on the surface of wheat seeds.

4.7.1.2 Incubation Methods

In this method, the seeds are examined after incubating them on some suitable media or substrate. Different techniques under this are:

- ***Blotter Method:*** This is the most convenient and efficient of all the incubation methods. In 1938, Doyer was first to adopt blotter method in seed health testing. The fungi are identified based on the habit characters in situ.
- ***Rolled Paper Towel Method:*** This method was developed by Warham (1990) to detect *Tilletia indica* effect on viability, germination, and vigor of wheat seed. Rolled paper towel method is used to know the effect of seed-borne inoculum on seed quality parameters, i.e., to carry out germination and vigor tests of apparently healthy and infected seed lots and also to see the effect of different seed treatments on seed-borne inoculum as per the International Seed Testing Association Rules (Anonymous 1996). Mainly, this method is used for the detection of *Fusarium* spp. in cereals (Sharma et al. 1987) and *Ascochyta* diseases in pea. The seed lot showing the higher seed vigor index is considered to be healthier (Abdul-Baki and Anderson 1973).
- ***2,4-D Method:*** The use of 2,4-D in the blotter test was first introduced by Neergaard (1956a, b) while testing cabbage seeds for *Phoma lingam*, although Hagborg et al. (1950) first used it in agar medium for the detection of *Colletotrichum lindemuthianum*, an anthracnose causing fungi on bean seeds. 2,4-D retards seed germination and seedling growth due to which seeds are not displaced, and the examination of pathogen becomes easy. The method has been recommended by ISTA (1966) as a standard test for a number of seed-borne pathogens.
- ***Deep-Freezing Method:*** Limonard (1968) was the first to use this method in seed health investigations. Deep freezing does not affect the fungi associated with the seeds as the imbibed seeds on moist blotters are killed.
- ***Agar Plate Method:*** In Northern Ireland, Muskette and Malone (1941) first used this method for seed health testing of flax seeds. This type of test helps in determination of type of inoculum and quantity of inoculum of the seed-borne fungi of a particular seed lot. There are some specific agar plate methods developed by various scientists like:
  - ***Oxgall-PDA*:** Miller et al. (1951) developed this medium which is suitable for detection of *Septoria* and *Fusarium* spp. and actually restricts the fungal colonies.
  - ***Peptone-PCNB Agar:*** Limonard (1968) found peptone-PCNB Agar medium to be the most suitable for *Fusarium* spp. associated with cereals.
  - ***Hold Fast Method:*** Mangan (1971) developed this method for detection of *Phoma betae* infection in sugar beet seeds with the naked eye.
  - ***Guaiacol-Agar Method:*** This selective procedure was developed by Kulik (1973) for detecting infection caused by *Pyricularia oryzae.*

4.7.1.3 Seedling Symptom Tests or Growing-On Tests

These tests help to observe the field performance of a seed lot in relation to seed-borne seedling diseases. Various method under this are:

- ***Hiltner's Brick Stone Method:*** It was developed by Hiltner in 1917 for field performance, giving information on seedling symptoms. It is also used for testing treated seeds.

- ***Sand Method:*** Endo (1961) was able to detect nonpathogenic and pathogenic isolates of *Rhizoctonia solani* from turfgrass by sowing seed on sterile quartz sand in culture plates watered with sterile water. The results obtained from sand media are more accurate and reproducible in comparison with "roll towel" tests especially in case of seed lots that are aged or heavily treated with chemicals.
- ***Standard Soil Method:*** This method was developed by Karlberg in 1974. Used soil is sterilized by heating at 95 °C for 20–30 min and can be used again and again for planting the seeds.
- **Test Tube Agar Method:** Khare, Mathur, and Neergaard in 1977 developed this method for the detection of *Septoria nodorum* in wheat grains. It can also be used for detection of *Colletotrichum* spp., *Fusarium* spp., and *Drechslera* spp. on various hosts especially in barley and legumes.

4.7.1.4 The Embryo Count Method

This special method used to separate the embryo for the detection of loose smut of wheat and barley has been devised by Hewett (1970). When the inoculum of a fungus, viz., *Ustilago nuda*, is located in the embryo, the separation of the embryo from the rest of the seed for microscopic observations is necessary (Rennie 1982). This method has been improved for avoiding the use of phenol and reducing the number of embryos examined per seed sample (Khanzada et al. 1989; Cappelli et al. 1993).

4.7.1.5 Staining Tests

Some staining compounds specific for fungal hyphae, i.e., trypan blue, aniline blue, rose bengal, etc., are used when inoculum is present in the internal tissues of the seed. In 1984, Welty and Rennie found the presence of typical mycelium of *Neotyphodium* spp. in perennial ryegrass, and tall fescue seeds were observed in a preparation obtained after seed softening in NaOH solution for about 15 hours and by crushing them under a coverslip on a microscope slide.

4.7.1.6 Serological Technique

Irwin (1987) was the first to report that detection of fungi by serological methods may be achieved only when monoclonal antibody techniques are employed. Monoclonal tests are known to detect seed-borne fungi of spruce (Mitchell 1988) and rice (Dewey 1992) and also *Pyrenophora graminea* (Burns et al. 1994) in barley.

4.7.2 Techniques Used for Detection of Seed-Borne Bacteria

Often, the percentage of infected seeds in a sample is so low that detection becomes extremely difficult in particular for seed-borne bacteria and seed-borne pathogens in general. Quick and readily applicable techniques which facilitate the recognition of the infected seeds in a sample easily are mentioned below.

4.7.2.1 Visual Separation of Infected Seed

Recognition and separation of infected seeds showing massive encrustation is relatively very simple. Many seeds which do not show massive encrustation will pass off undetected by visual separation method. The seeds which cannot be recognized as infected by routine examination under ordinary light conditions show tiny diffused fluorescent patches when scanned under the near ultraviolet light. According to Wharton (1967), the selection of all fluorescent seeds from large samples for bacteriological testing can markedly increase sensitivity of the testing method by concentrating infected beans into small subsamples and increases the probability of their detection. Parker and Dean (1968) found that bean seeds infected with *Pseudomonas phaseolicola* did not show external symptoms unless there was a very massive concentration.

4.7.2.2 Phage Method

Katznelson and Sutton (1951) first detected and identified seed-borne *Xanthomonas campestris* pv. *phaseoli* or *Pseudomonas phaseolicola* by this method. The technique is based on the multiplication and increase in the number of specific bacteriophage particles in the presence of a susceptible bacterium.

4.7.2.3 Serological Technique

Serology as a method for bacteria detection was first employed, in 1918. It is based on in vitro reactions between antigens and antibodies (Marcinkowska 2002).

Agglutination, precipitation, and immunodiffusion tests belong to the earlier serological methods based on polyclonal antibodies still in use for certain bacteria (Ball and Reeves 1992). Guthrie et al. (1965) developed serological technique for detection of halo blight (caused by *Pseudomonas phaseolicola*) in bean seed samples.

- *Enzyme-Linked Immunosorbent Assay.*

Enzyme-linked immunosorbent assay called ELISA was first adapted for plant viruses' identification by Clark and Adams (1977). In 1981, Clark developed the principles of this method. Both polyclonal and monoclonal antibodies can be applied for ELISA, but monoclonal antibodies improved efficacy of ELISA in detection of seed pathogens (Fox 1993). There are many different forms of ELISA (Ball and Reeves 1992), but three of them are majorly followed: direct (double antibody sandwich DAS-ELISA), indirect, and competitive (Lange 1986). The direct test is the most often used in the seed health assays (Lange 1986; Kazinczi and Horvath 1998).

Candlish et al. (1988) first developed highly specific monoclonal antibodies to *Pseudomonas syringae* pv. *pisi* (causal organism of bacterial blight on pea seeds) for both indirect and competitive ELISA assays to distinguish between strains of this bacterium. Moreover, first commercial kit for detection of the bacterial seed-borne infection was prepared and marketed by Ball and Reeves (1991). Later, Rajeshwari et al. (1998) developed a sensitive, specific, and rapid ELISA technique for detection of *Ralstonia solanacearum* isolates from tomato seeds.

- *Immunofluorescence Microscopy.*

Immunofluorescence microscopy is a second important assay and highly recommended immunodiagnostic method for detection of seeds infested with bacteria (Van Vuurde 1997). Indirect (Malin et al. 1983) and direct (Franken and Van Vuurde 1990) immunofluorescence cell staining involves microscopic detection of an antigen under ultraviolet light after staining it with homologous antibody conjugated with a fluorescent dye (e.g., fluorescein isothiocyanate). Fluorescence is proportional to the concentration of bacteria in the seed preparation. For example, *Xanthomonas campestris* pv. *phaseoli* was identified in bean seed by indirect method, whereas *Pseudomonas syringae* pv. *phaseolicola* was detected by direct immunofluorescence. The same method was also used for *Clavibacter michiganensis* subsp. *michiganensis*.

- *Immunofluorescence Colony-Staining (IFC) Method.*

Van Vuurde (1997) described the immunofluorescence colony-staining (IFC) method for routine indexing and quantitative determination of field thresholds of pathogenic seed-borne bacteria. The IFC assay is more sensitive and more specific than traditional isolation and the earlier discussed serological tests.

4.7.2.4 Growing-On Test

The seedlings are grown out of infected seeds under conditions optimal for the manifestation of disease symptoms on the growing seedlings. Srinivasan et al. (1973) developed and standardized this method for the detection of *Xanthomonas campestris* pv. *campestris* in a seed sample of cauliflower. However, the growing-on tests for detection of bacteria and viruses have been replaced by the enzyme-linked immunosorbent assay since 1985 in several countries (Dinant and Lot 1992).

4.7.2.5 Indicator Test

The main objective of this method is to produce viral symptoms on healthy seedlings or mature plants (used as indicator plants) by using inoculum of infected or contaminated seeds. The inoculum from seeds slightly infected by *Xanthomonas campestris* pv. *phaseoli* or *Xanthomonas phaseoli* var. *fucans* when inoculated in indicator plants by hypodermic injection gave more sensitive results than serological techniques (Saettler 1971, 1973, 1974; Andersen and Young 1973; Schuster and Coyne 1975).

4.7.3 Techniques Used for Detection of Seed-Borne Viruses

4.7.3.1 Dry Examination

A close visual inspection of the seed may give an indication of the presence of virus in certain seeds. Phatak (1974) found that in 13 genera, no less than 16 seed-borne viruses may leave a clue of their presence in the seed.

4.7.3.2 Biological Methods

These require extensive greenhouse facilities and divided into two subheadings:

- ***Growing-On Tests:*** Symptoms of *Bean common mosaic virus* (BCMV) in bean, *Bean yellow mosaic virus* (BYMV) in pea, *Soybean mosaic virus* (SMV) in soybean, etc. may appear by growing seed in moderate conditions of temperature and light, but in some viruses, e.g., *Barley stripe mosaic virus* (BSMV) and *Lettuce mosaic virus* (LMV), specific conditions of light and temperature are important.
- ***Infectivity Test:*** Test plants are inoculated with extract of test seedlings, germinated or even ungerminated seed. Pelet and Gagnebin (1963) and Pelet (1965) for the first time tested LMV in lettuce seed by macerating them with a buffer solution and by inoculating seedlings of *Chenopodium quinoa*. *Bean common mosaic virus* (BCMV) in bean seeds can be tested by this biological technique.

4.7.3.3 Biochemical/Histochemical Methods

Phatak (1974) demonstrated viral inclusions by staining infected tissues with 1% aqueous phloxine solution. This method is based on the fact that viral infections cause biochemical and physiological changes in host tissues.

4.7.3.4 Serological Methods

Modifications of the original ELISA described by Clark and Adams (1977) are necessary for effectiveness of detection of seed-borne virus. Frison et al. (1990) antecedently stated that ELISA is a preferred method for indexing 25 out of the 35 seed-transmitted viruses of legumes listed in the technical guidelines ensuring safe movement of legume germplasm internationally. Immunoassays such as the biotin/avidin ELISA and the enzyme-linked fluorescent assay (ELFA) were equally effective and superior as standard ELISA for detection of LMV (*Lettuce mosaic virus*) in lettuce seeds. Dot-ELISA (dot blot, dot immunobinding or spot immunodetection) is a newer modification of the ELISA technique (Lange 1986; Stead 1992). This is an indirect method which was found to be slightly more sensitive for *Pea seed-borne mosaic virus* than other ELISA techniques (Lange 1986).

- ***Gel Diffusion Techniques:*** Macromolecules of viral protein antigens and corresponding antibodies can react within agar substrate which is permeable to their diffusion.
  - ***Single Diffusion Test:*** In this test, the antiserum is mixed with the agar before it solidifies. The antigen (virus) has to diffuse and react with antibodies present throughout the agar.
  - ***Double Diffusion Test***: In this test, both reactants have to diffuse through agar from separate depots. For the first time, Scott (1961) followed by Hamilton (1965) employed this method for the detection of *BSMV* and Phatak (1974) for the detection of *TMV* in tomato seeds.
- ***Agglutination Technique:*** In this test, the antigen/antibody reaction results in agglutination or clumping of a particular antigen (Lyons and Taylor 1990). There are some specific agglutination techniques developed by various scientists like:

- ***Chloroplast Test:*** Jermoljev and Chod (1966) employed this technique for the first time for the detection of *BCMV* in germinated bean seeds in which clumping of chloroplasts indicates presence of virus.
- ***Latex Test:*** Phatak (1974) primarily used latex agglutination test for detecting *BSMV* in barley, soybean mosaic virus in soybean, BCMV in bean, and *Bean yellow mosaic virus* in broad bean. He found this technique as one of the most promising for routine work.

4.7.3.5 Biophysical Methods

The presence of virus in seed can be detected directly by electron microscopy or indirectly by radiography of seed with X-rays.

- ***Electron Microscopy:*** An electron microscope can be used in seed health testing for viruses. Previously, Gold et al. (1954) observed particles of *Barley stripe mosaic virus* (BSMV) by using an electron microscope for barley seeds. After that, Phatak (1974) found particles of *Bean common mosaic virus* (BCMV) in bean seeds and *SoyMV* and *BSMV* in the plumules, respectively, under electron microscope.
- ***Immunosorbent Electron Microscopy:*** By using electron microscopy, it is difficult to identify the virus specifically, since several viruses belong to the same morphological class. This difficulty can be eliminated by using immunosorbent electron microscopy (ISEM), a combination of serology and electron microscopy.
- ***Contact Radiography with "Soft" X-Rays:*** Phatak and Summanwar (1967) recorded for the first time that on the basis of decreased opacity to X-ray penetration, it was possible to distinguish between barley seeds infected with *BSMV* and runner bean (*Phaseolus multiflorus*) seeds infected with *Runner bean mosaic virus* (RBMV).

4.7.4 Expansion of Molecular Detection of Seed-Borne Microflora

4.7.4.1 Probes

Nucleic acid probes have been widely used in plant pathology as it makes identification correct and fast, but only with pure colonies of pathogens. Several attempts were made to use this method for bacteria (Schaad et al. 1989), fungi (Reeves 1995), and viruses (Lange 1986), but probes are not applied in routine seed health testing because when seed extracts are probed directly, contaminating DNA may cause problems in accurate detection of organisms. In this case, it is better to extract organisms from seeds and purify them before the probes are applied (Maude 1996).

4.7.4.2 Polymerase Chain Reaction

The polymerase chain reaction (PCR) (Mullis and Faloona 1987) is a method which enables amplification and multiplication of the target sequence of DNA up to a millionfold (Saiki et al. 1988; Oliver 1993) due to its specificity, speed, and sensitivity.

In case of fungi, the PCR techniques have been mainly used for characterization and identification of these organisms (Reeves 1995), and there are few earliest examples of detection and identification of fungi in seeds, e.g., *Phomopsis* species in soybean seeds (Jaccoud-Filho and Reeves 1993). First and foremost, Smith et al. (1996) applied the BIO-PCR method for identification of *Tilletia indica*, a seed-borne pathogen of wheat. RAPD was not suitable for direct detection, so it was employed with the internal transcribed spacer (ITS) region of the nuclear ribosomal unit by Stevens et al. (1997) for development of a multiplex PCR seed health test which was able to detect and differentiate *Pyrenophora* spp. pathogenic to barley.

The PCR techniques have been applied for detection of a few bacterial species, e.g., *Pseudomonas syringae* pv. *phaseolicola* and *P. syringae* pv. *pisi* in bean and pea seeds, respectively (Prosen et al. 1991, 1993; Rasmussen and Wulff 1991; Reeves et al. 1994), and *Pantoea* (*Erwinia*) *stewartii* in maize seeds (Blakemore and Reeves 1993). Schaad et al. (1995) earliest developed a highly sensitive PCR technique named BIO-PCR to detect *Pseudomonas syringae* pv. *phaseolicola* in bean seeds.

It was first reported by Fox (1993) that the majority of plant viruses has RNA genomes and as such is unsuitable for PCR. To prevail over this incongruity, silica capture reverse transcriptase polymerase chain reaction (SC-RT-PCR) (Boom et al. 1990) and the immune capture reverse transcriptase polymerase chain reaction (IC-RT-PCR) (Levy and Hadidi 1991; Nolasco et al. 1993; Candresse et al. 1995) were developed to detect seed-borne plant viruses. The IC-RT-PCR was first applied by Van der Vlugt et al. (1997) for LMV detection in seed samples. The IC-RT-PCR was also used to detect *Cherry leaf roll nepovirus* (CLRV) isolates and strains from the seeds of *Betula pendula* by direct sequencing of their PCR products (Buttner et al. 1997).

As compared to conventional PCR, real-time PCR has several key advantages that potentially make it more acceptable for use in routine seed testing (Walcott 2003). Significant benefits can be realized by combining real-time PCR with the other PCR modifications mentioned above (MCH-PCR, IMS-PCR, BIO-PCR). Real-time PCR seed detection assays have been reported for *A. avenae* subsp. *citrulli* in watermelon seeds and *Microdochium nivale* in wheat (*Triticum* spp.) seeds (Taylor et al. 2002). It is likely that more real-time PCR seed assays will be developed as the technology becomes more affordable.

DNA chips or microarrays represent another DNA-based detection assay that may be applied to test seeds for pathogens (Walcott 2003). Currently, few DNA-chip seed detection assays have been developed (Fessehaie et al. 2001). However, it is envisioned that this technology will be more widely employed for routine seed testing in the future.

4.8 Conclusion

Seed is the basic unit in crop production. A large number of crops depend on true seeds for their next progeny. Science of seed pathology is now-a-days in much focus, since sowing of a poor-quality seed leads to ensured loss in plant stand and crop yields and ultimately can pose a threat to the "food security" for ever-growing global population. Seed-related aspects are continuing to keep puzzling the researchers since long back. The science of seed pathology has traveled a long distance in the history from the rare documentation about the seed treatments with cow ghee, honey, cow urine, ashes, etc. to era of well-standardized seed coating, bio-priming, and molecular techniques based on detection and diagnosis of seed-borne pathogens and diseases. The systematic applications of these techniques lead to the ensured availability of healthy and disease-free quality seed.

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Part III

Diagnosis & Detection of Seed-Borne Pathogens

Diagnosis and Detection of Seed-Borne Fungal Phytopathogens

5

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Abstract

Food losses due to crop infections caused by different pathogens such as bacteria, viruses and fungi are persistent issues in agriculture for centuries across the globe. The timely detection and appropriate identification of casual agents associated with diseases of crop plants or seeds are considered to be the most important issue in formulating the management strategies. Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases. Specificity, sensitivity, speed, simplicity, cost-effectiveness and reliability are the main requirements for the selection of seed health test methods. Examples of frequently used seed assays include visual examination, selective media, seedling grow-out and serological assays which, while appropriate for some pathogens, often display inadequate levels of sensitivity, specificity and accuracy. Polymerase chain reaction (PCR) has emerged as a tool for the detection of microorganisms from diverse environments. Thus far, it is clear that nucleic acid-based detection protocols exhibit higher level of sensitivity than conventional methods. Unfortunately, PCR-based seed tests require the extraction of PCR-quality DNA from target pathogens in backgrounds of saprophytic organisms

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and inhibitory seed-derived compounds. The inability to efficiently extract PCR-quality DNA from seeds has restricted the acceptance and application of PCR for the detection of seed-borne pathogens. To overcome these limitations, several modified PCR protocols have been developed including selective target colony enrichment followed by PCR (Bio-PCR). These techniques seek to selectively concentrate or increase target organism populations to enhance detection and have been successfully applied for detecting fungi in seed. Ultimately, improved protocols based upon PCR, ELISA, etc. will be available for the detection of all seed-borne pathogens and may supersede conventional detection methods. This chapter provides a comprehensive overview of conventional and modern tools used for the early detection and identification of seed-borne fungal pathogens.

5.1 Introduction

Seed-borne pathogens possess a serious threat to seedling establishment. Close association of the pathogens with seeds facilitates their long-term survival, introduction into new areas and widespread dissemination. Under such conditions, elimination is the most effective disease management strategy accomplished by using seed detection assay to screen and reject infested seed lots before sowing/planting or its distribution to the farmers or seed growers. Transboundary spread of pathogens is a major concern today. Precise detection methods are essential for seed-borne pathogens to support seed health strategies. While choosing a method, it is essential to see that it is reliable, less time-consuming, cost-effective, reproducible and sensitive. The conventional seed health testing methods are being used in the identification of fungus up to species level. The considerable advancement in molecular biology has facilitated rapid identification/detection of seed-borne pathogens. Over 100 years of seed health studies, many new methods were developed, or older methods were modified, but all of them used for the detection and identification of seed-borne organisms have to fulfil six main requirements (Ball and Reeves 1991):

(i) ***Specificity*** – the ability to distinguish a particular target organism from others occurring on tested seeds.
(ii) ***Sensitivity*** – the ability to detect organisms at low incidence in seed stocks.
(iii) ***Speed*** – less time requirements, to enable prompt action against the target pathogen(s).
(iv) ***Simplicity*** – minimization of a number of examination stages to reduce error and enable testing by a staff not necessarily highly qualified.
(v) ***Cost-effectiveness*** – costs should determine acceptance to the test.
(vi) ***Reliability*** – methods must be sufficiently robust to provide repeatable results within and between samples of the same stock regardless who performs the test.

5.2 Why Detection of Seed-Borne Fungal Pathogens is Important?

- Seed-borne fungal pathogens present a serious threat to seedling establishment and hence may contribute as potential factor in crop failure.
- Seeds not only facilitate the long-term survival of these pathogens but also may act as a vehicle for their introduction into newer areas and their widespread dissemination.
- Seed-borne fungal pathogens are able to cause catastrophic losses to food crops and hence directly linked to the food security.
- Unlike infected vegetative plant tissues, infested seeds can be asymptomatic, making visual detection impossible.
- Additionally, fungal pathogen's populations on seeds may be low, and the infested seeds may be non-uniformly distributed within a lot.

5.3 Detection Methods for Seed-Borne Fungal Pathogens

The following methods are used to detect seed-borne fungal pathogens which include conventional and modern methods.

5.3.1 Conventional Detection Methods

5.3.1.1 Visual Examination of Dry Seeds

The first step of the detection of seed-borne pathogens is examination of dry seeds with unaided eye (naked eye) or with the magnifying glasses (hand lens). In certain cases, infected seeds exhibit different characteristic symptoms produced by various seed-borne fungal pathogens on seed surface, viz. seed rot, seed necrosis, shrunken seed, seed discolouration, shrivelling, etc. (Table 5.1 and Fig. 5.1). Besides these symptoms, dry seeds are examined for the presence of admixtures such as sclerotia, fungal fructification such as pycnidia and acervuli, smut balls and smut sori, etc. In this method stereoscopic microscope, hand lens or naked eye can be used for a sample consisting of 400 or more seeds. By this examination some additional significant risks can also be eliminated, e.g. weed seed contaminants, insect pests and abnormal seeds. Seed may be soaked in water or other liquids to make pathogen structures, e.g. pycnia, and symptoms, i.e. anthracnose, on the seed coat more visible.

Visual examination method may be coupled with automatic devices that sort seeds based on visuals of physical characteristics (Paulsen 1990; Walcott et al. 1998) to reduce seed lot infestation. But, as a limitation, these systems usually have low detection sensitivity, which makes these devices less useful in decision-making system for rejection of seed lots. Additionally, seeds infested by fungi, bacteria and viruses may display no macroscopic symptoms, making visual or physical inspection of seeds useless as a detection assay.

Table 5.1 Visual sign/symptoms of some major seed-borne fungi on various crop seed

S. no.	Crop	Visual sign or symptom on seed	Possible fungi associated	References
1.	Barley	Scald symptoms	*Rhynchosporium secalis*	Lee et al. (1999)
2.	Carrot	Seed rot	*Alternaria radicina*	Gaur (2011)
3.	Celery	Pycnidia embedded in the seed coat	*Septoria apii*	Horst (2008)
4.	Cereals	Normal seed is replaced by sori of spores	Smut, bunt or ergot in cereals	Warham et al. (1996)
5.	Chick pea	Small and wrinkled seed	*Fusarium oxysporum* f. sp. *ciceri*	Khare (1996)
		Ashy brown discolouration in seeds	*Ascochyta rabiei*	Khare (1996)
		Blackish seed coat	*Alternaria alternata*	Khare (1996)
		Reduction in seed size	*Ascochyta rabiei*	Gaur (2011)
6.	Chilli	Acervuli and microsclerotia	*Colletotrichum dematium*	Kumar et al. (2004)
7.	Coriander	Hypertrophied seed	*Protomyces macrosporus*	Khare (1996)
8.	Crucifers	Reduction in seed size and seed rot	*Phoma lingam*	Gaur (2011)
		Shrivelling	*Alternaria brassicae*, *A. raphani* and *A. alternata*	Rude et al. (1999)
9.	*Dolichos lablab*	Red discolouration around micropyle (red nose)	*Stemphylium botryosum*	Gaur (2011)
		Brown spot	*Colletotrichum lindemuthianum*	Gaur (2011)
10.	Lentil	Ashy brown discolouration in seeds	*Ascochyta fabae* f. sp. *lentis*	Khare (1996)
11.	Maize	White streaks with black spore masses near the tips	*Nigrospora* sp.	Agarwal and Sinclair (1997)
		Seeds exhibit white streaks	*Fusarium moniliforme*	Khare (1996)
		Seed rot	*Fusarium graminearum*	Gaur (2011)
12.	Onion	Seed rot	*Alternaria porri*	Gaur (2011)
		Shrunken seeds	*Peronospora destructor*	Gaur (2011)
13.	Pea	Brown spot	*Ascochyta pisi*	Gaur (2011)
		Seed rot	*Mycosphaerella pinodes*	Gaur (2011)
14.	Peanut	Speckles	*Cylindrocladium parasiticum*	Randall-Schadel et al. (2001)
15.	Rice	Light pink discolouration	*Fusarium graminearum*	Sachan and Agarwal (1995)
		Ash grey discolouration	*Alternaria alternata*	Sachan and Agarwal (1995)
		Black discolouration, dark brown spots and light to dark brown dot-like spots	*Helminthosporium oryzae* [*Cochliobolus miyabeanus*]	Sachan and Agarwal (1995)
		Light brown discolouration	*Sarocladium oryzae*	Sachan and Agarwal (1995)

(continued)

Table 5.1 (continued)

S. no.	Crop	Visual sign or symptom on seed	Possible fungi associated	References
16.	Sesame	Hyphae and sclerotia on seed coat	*Macrophomina phaseolina*	Khare (1996)
17.	Sorghum	Completely deformed	*Acremonium* sp.	Agarwal and Sinclair (1997)
		Shrunken seeds	*Sclerospora sorghi*[a]	Gaur (2011)
18.	Soybean	Purple stain	*Cercospora kikuchii*	Murakishi (1951) and Khare (1996)
		Fine cracks and mould, starting near the hilum	*Phomopsis longicolla*	Li (2011)
19.	Wheat	Bunted seed	*Tilletia tritici*	Warham (1986)
		Shrivelled rough scabby appearance	*Fusarium* sp.	Warham et al. (1996)
		Black point on seed	*Alternaria alternata*	Khare (1996)

[a]Not seed-borne but affect seed

Fig. 5.1 Visual symptoms caused by fungal pathogens on seeds after harvesting (**a**–**d**) and in standing crops (**e**–**h**): Karnal bunt of wheat (**a**), false smut of paddy (**b**), kernel smut/bunt of paddy (**c**–**d**), loose smut of wheat (**e**), ergot of pearl millet (**f**), green ear disease of pearl millet (**g**), smut disease of pearl millet (**h**)

5.3.1.2 Microscopic Examinations

(a) **Examination of Seed Washings**

This method is used to detect seed-borne pathogens which are loosely present on the seed surface. This method is mostly used for the detection of fungi causing smuts, bunts, downy mildew, powdery mildew and rust with the important exception of loose smut of wheat and barley which are internally seed-borne diseases. For seed washing test, seed samples (50 seeds) are placed in test tubes containing sterile distilled water (10 ml) and a few drops (10–20) of 95% ethyl alcohol or a detergent. The sample tubes are agitated in a mechanical shaker for 10 min. The aqueous

suspension is then centrifuged at 1000 rpm for 10 min. The supernatant is poured off and the pellet is re-suspended in 2 ml of sterile water. Spores or fungal structure present in the suspension can be viewed by examining a few drops of the suspension under the light microscope.

(b) **NaOH Seed Soak Method**

The NaOH seed soak method was first used by Agarwal and Srivastava (1981). This method is applied for the detection of Karnal bunt of wheat and bunt (kernel smut) of paddy. In this method seeds are soaked in 0.2–0.3% NaOH solution for 24 h at 25–30 °C. Next day the solution is decanted and the seeds are thoroughly washed in tap water. After washing, the seeds are spread over blotter paper so that the excess moisture is absorbed by blotter. Now the seeds are examined visually. The wheat seeds showing black to shiny black discolouration may contain Karnal bunt infection of *Tilletia indica.* This may be confirmed by rupturing suspected seed with a fine needle in a drop of water, the bunt spores (teliospores) will be released, if the suspected seed is infected. Similarly, the infection in paddy seeds due to bunt or kernel smut disease of paddy caused by *Tilletia barclayana* can also be detected. Likewise, by treating the rice seeds with NaOH (0.2%), the infection by *Trichoconiella padwickii* could be inferred by the change of colour of the diseased portion of infected seeds to black (Singh and Maheshwari 2001).

(c) **Whole Embryo Count Method**

This method is used when seed-borne infection is deep seated in the seed tissues such as embryo in case of loose smut of wheat and barley. The embryo count method was first used by Skvortzov (1937) for detecting loose smut pathogen *Ustilago nuda* var. *tritici*. He dissected the embryos, macerated them with NaOH and then stained them with aniline blue. This method is completed in 3–4 days. This method was modified by Agarwal et al. (1978) as follows (Fig. 5.2):

5.3.1.3 Incubation Methods

(a) **Testing on Agar Media:** In agar tests seeds are incubated on agar media for a particular length of time and optimum temperature under alternating light and dark cycles. The associated fungi are detected based on their morphological and habit characters on seed surface and colony characters on the medium. It is used to detect *Alternaria*, *Bipolaris*, *Curvularia*, *Fusarium*, etc. in infected seeds.

(b) **Blotter Testing:** Doyer (1938) and de Temp (1953) were first to adopt blotter paper method in seed health management. This test is used to detect infection of seeds, and in certain cases, infection of the germinated seedlings can also be detected by this method. Blotter method is the most widely used seed health assay. Mainly this method is of two types:

(i) **Standard Moist Blotter (SMB) Method**

In the standard blotter test, seeds are sown in Petri dishes containing 1–3 layers of water or buffer-soaked absorbing (blotting) paper or cellulose pads

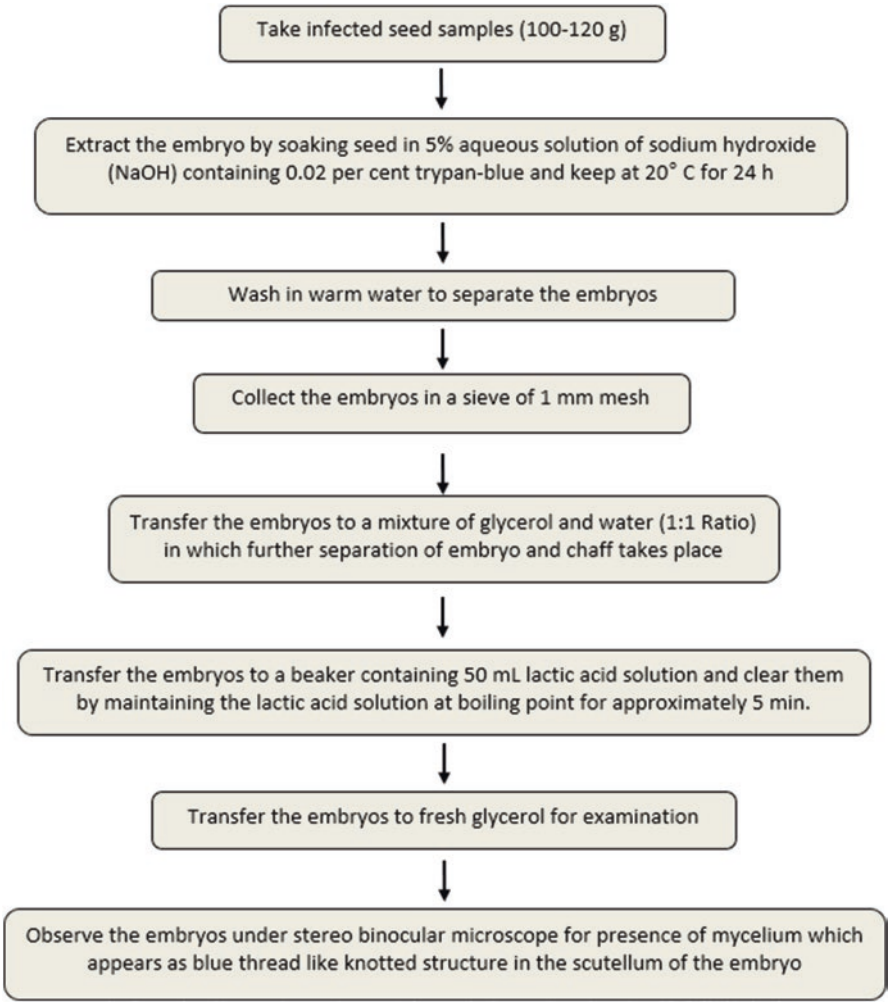

Fig. 5.2 General protocol for whole embryo count method for detection of loose smut of wheat

for a couple of days depending on the fungus and type of seed tested (Marcinkowska 2002). In general, 10–20 non-sterilized seeds (depending on the seed size) are placed equidistant from each other in Petri dish and incubated at 25 ± 2 °C with alternate cycles of 12 h of light and 12 h of darkness for 7–10 days. In the blotter test, seeds are subjected to conditions that enable pathogen growth and expression during the incubation period (Fig. 5.3). After the incubation period, the seeds are examined under a stereomicroscope for the presence of fungal colonies, and their characteristics are recorded for the identification of the fungal pathogens.

The seed must be surface-sterilized prior to its placement on blotter paper in Petri dish, if the internally seed-borne fungal pathogens are to be

Fig. 5.3 Incidence of seed mycoflora on pea seeds under blotter testing method after 10 days of incubation

detected. Seeds may be immersed in a NaOCl solution containing 1% chlorine for 10 min or in 1.7% NaOCl solution for 1 min followed by immersion in 70% chlorine for 10 min (ISTA 1966). The germination of seeds may be obstructed by wetting the blotting paper with 0.1–0.2% 2,4-D. This procedure has been used for the detection of *Leptosphaeria maculans* (anamorph *Phoma lingam*) in crucifer seeds (Hewett 1977) and for routine seed health testing of common bean and soybean (Dhingra et al. 1978).

(ii) **Deep Freezing Blotter (DFB) Method**

The DFB method is used to detect a wide range of fungi which are able to grow easily from seeds in the presence of humidity. After plating seeds as described in the SMB method, the Petri dishes are incubated at 20 ± 2 °C for 24 h and then transferred to a − 20 °C freezer for 24 h followed by incubation at 20 ± 2 °C for 5 days under cool white fluorescent light with alternating cycles of 12 h light and 12 h darkness. Pure cultures are obtained through hyphal-tip and single-spore isolation techniques and maintained on carrot potato agar (CPA) slants for further studies. Fungi are identified using cultural, biochemical, macromorphological and micromorphological characteristics as described by Raper and Fennel (1965), Booth (1971), Ellis (1971) and Domsch et al. (1980).

The percentage of seed infection in each sample and the percentage of infection in each region are determined by the following formulae:

$$\text{Mean rate of seed infection} = \frac{\text{Number of seeds on which a fungal species identified}}{\text{Number of seeds tested}} \times 100$$

$$\text{Mean of regional infection} = \frac{\text{Frequency of sample on which a fungus identified}}{\text{Number of samples collected}} \times 100$$

For species-level identification, the fungi are isolated on potato dextrose agar (PDA) and maintained at 24 ± 1 °C for 7–10 days. The identification is conducted using colony colour, colony texture pattern, arrangement of spore on the conidiophores, spore shape and size (Watanabe 2002; Leslie and Summerell 2006; Utobo et al. 2011).

The blotter method has been coupled with scanning electron microscopy (SEM) for the detection of seed-borne fungi (Alves and Pozza 2009). The seeds of common bean (*Phaseolus vulgaris* L.), maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) were submitted to the standard blotter test. The specimens were prepared and observed with the standard SEM methodology. It was possible to identify *Fusarium* sp. on maize, *C. gossypii* var. *cephalosporioides* and *Fusarium oxysporum* on cotton and *Aspergillus flavus*, *Penicillium* sp., *Rhizopus* sp. and *Mucor* sp. on common bean (Alves and Pozza 2009).

(c) **Seedlings Symptoms Test and Grow-Out Test**

Seedlings symptoms test is based on the characteristic symptoms produced by seed-borne fungi on growing seedlings under controlled conditions, whereas in grow-out test, plants are grown beyond the seedling stage in near-optimum conditions of temperature and moisture in sterile medium, i.e. sand, and water-agar medium, and the seedlings/plants are observed for symptoms of the fungal pathogens. It can facilitate the detection of a number of fungal pathogens associated with seed rotting and other symptoms at seedling stage, e.g. fungal pathogens causing seedling diseases as *Alternaria*, *Bipolaris*, *Fusarium*, *Pyricularia*, etc. This method involves the planting of a certain number of seeds, preferably on sterile soil for determining the number of infected plants and calculating the percent infected plants out of the total number of seed sown. These test results are helpful in assessing field performance and estimating the number of infection loci/unit area, if the seed lot under investigation is used for cultivation by farmers. Infection of soybean seeds by *Colletotrichum truncatum* was detected by this method (Dhingra et al. 1978). This method is very effective in the case of non-cultivable obligate pathogens causing downy mildew diseases. However, it requires large greenhouse space, and also it is time-consuming, making it unsuitable for testing a large number of seed lots. There are a number of seedling symptoms and grow-out tests as follows:

(i) **Test Tube Agar Method**

This method was developed by Khare, Mathur and Neergaard in 1977. It is used for the detection of *Septoria nodorum* in wheat seeds and is very

useful for assaying the small quantity of high cost material. In this method infection of root can also be examined. Fungal pathogens of cereals like *Drechslera* sp., *Bipolaris* sp. and *Septoria* sp. can be easily detected. Steps used in procedure are as follows:

1. 15 ml water agar is taken in test tube, sterilized and solidified with a slight slant.
2. One seed is sown in each test tube and incubated at 28 ± 1 °C with 12 hours alternating cycles of light and darkness.
3. Seedlings are examined after 14 days for the typical symptoms of disease in the coleoptiles.
4. The symptoms can be easily studied being visible on roots as well as on green parts.

(ii) **Hiltner's Brick Stone Method**
It was developed by Hiltner in 1917. Sterile crushed brick stone with a maximum piece size of 3–4 mm is used to fill in plastic pots up to ¾th of their capacity. The crushed brick stone in the pot is saturated with water and seeds are placed 1 cm deep. The pots are kept in darkness at room temperature, and observations for disease symptoms are recorded after 2 weeks by removing the seedlings. It is a good method for testing field performance giving information on seedling symptoms. It is also used for testing treated seed.

(iii) **Sand Method**
This method is similar to Hiltner's brick stone method except that in place of sterile crushed brick stone, sterilized sand is used.

(iv) **Standard Soil Method**
A pre-sterilized uniform soil mixture containing four parts clay, six parts peat and essential amount of fertilizer is filled in plastic multi-pot trays. After sowing the seed, appropriate moisture should be maintained. The symptoms are observed after incubation for 2–4 weeks depending on the kind of seed and temperature.

(d) **Selective Media**

It is a direct method of seed testing in which seed-borne pathogens are allowed to grow on specific media. The use of selective media for the detection of pathogens is more reliable than blotter or agar method. This can be done by directly plating surface-sterilized seed samples or seed wash liquid onto artificial media, followed by adequate incubation under favourable conditions. Once a fungal pathogen is isolated, it can be identified by its cultural, morphological or biochemical characteristics. Selective artificial media are developed that use antibiotics, fungicides, selected carbon and nitrogen sources and other inhibitory compounds to retard the growth of non-target microflora while allowing the target pathogen to grow. For example,

potato dextrose agar is useful for the detection of *Septoria nodorum* in wheat, while PCNB agar is a selective medium for the detection of *Fusarium* species in cereals. The list of some selective and semi-selective media for different seed fungi is given in Table 5.2.

Table 5.2 General/selective media and temperature requirements that favour the development of seed-borne fungal pathogens

S. no.	Name of seed-borne fungal pathogen	Nutrient medium	Incubation temp. (°C)	References
1.	*Botrytis cinerea*	Selective media: *Botrytis* selective medium (BSM) and *Botrytis* spore trap medium (BSTM)	25 °C	Edwards and Seddon (2001)
2.	*Botrytis cinerea*	Potato dextrose agar (PDA)	20–22 °C	Mirzaei et al. (2008)
3.	*Botryodiplodia theobromae*	PDA	28 °C	Fu et al. (2007)
4.	*Lasiodiplodia theobromae*	Selective medium	25 °C	Cilliers et al. (1994)
5.	*F. oxysporum* f. sp. *niveum*	PDA/lima bean agar	25 °C	Zhang et al. (2005)
6.	*F. oxysporum* f. sp. *cucurbitae*	*Fusarium* selective medium (FSM)	25–37 °C	Mehl and Epstein (2007, 2008)
7.	*F. solani* f. sp. *cucurbitae*	*Fusarium* selective medium (FSM)	22 °C	Mehl and Epstein (2007)
8.	*Trichoconiella padwickii*	Semi-selective media	28–30 °C	Muthaiyan (2009)
9.	*Fusarium graminearum*	Semi-selective media (NSA, SRA-FG)	25–28 °C	Segalin and Reis (2010)
10.	*Fusarium* species	Semi-selective medium (MGA 2.5 + carnation leaves)	25 °C	Thompson et al. (2013)
11.	*Exserohilum turcicum*	Semi-selective medium (DRR-Reis)	25 ± 2 °C	De Rossi and Reis (2014)
12.	*Alternaria brassicicola* (crucifer seeds)	Semi-selective media (CW medium)	24 °C	Wu and Chen (1999)
13.	*Fusarium* species in cereals	Dichloran chloramphenicol peptone agar (DCPA)	25 °C	Andrew and Pitt (1986)
14.	*Phoma betae*	Hold fast method	20 °C	Mangan (1971)
15.	*Fusarium moniliforme*	Modified Czapek's dox agar medium (MC_ZA)	26–28 °C	Agarwal and Singh (1974)
16.	*Pyricularia oryzae*	Guaiacol agar	25 °C	Kulik (1975)
17.	*Stagonospora nodorum*	SNAB (*Stagonospora nodorum* agar for barley)	20 °C	Cunfer and Manandhar (1992)
18.	*Septoria nodorum* [*Stagonospora nodorum*]	Selective media SNAW	20 °C	Manandhar and Cunfer (1991)

(continued)

Table 5.2 (continued)

S. no.	Name of seed-borne fungal pathogen	Nutrient medium	Incubation temp. (°C)	References
19.	*F. graminearum*	Toxoflavin-based selective medium	25 °C	Jung et al. (2013)
20.	*Curvularia lunata*	CS medium with 200 ppm carbendazim +200 ppm streptomycin and CR medium with 200 ppm carbendazim +200 ppm rifampicin	25 °C	Deshpande (1993)
21.	*Rhynchosporium secalis*	Lima bean agar medium	15–20 °C	Lee et al. (1999)
22.	*Fusarium* species in cereals	PCNB agar	22–25 °C	Pastircak (2007) and Alborch et al. (2010)

5.3.2 Serological Detection Techniques

The seed-infecting fungal communities may comprise the saprobes which can grow rapidly over the target fungal pathogens. These fast-growing saprobes arrest their isolation; and examination of their morphological characteristics becomes difficult and confusing. In the case of rice seed-borne pathogens, such situation exists, where about 30 fungal phytopathogens infecting rice have been reported to be seed-borne (Mew et al. 1988). Additionally, the presence of very closely related strains, race or even fungal species on the seeds makes the detection morphologically almost impossible. Therefore, more sensitive techniques such as immunoassay and nucleic acid-based protocols are needed to overcome this issue. Since pure culture of the pathogens is not needed in serological detection protocols, these techniques could be applied to detect biotrophic as well as necrotrophic seed-borne pathogens (Mancini et al. 2016).

After the first use of enzyme-linked immunosorbent assay (ELISA) by Clark and Adams (1977), employed for successful detection of plant viruses, this technique has been widely adopted and modified based on requirement of the assays (Fang and Ramasamy 2015). This serological method is used for the identification of diseases based on antibodies and colour change in the assay. Serological assays depend on antibodies generated against specific antigens of plant pathogens. The antibodies bind specifically to its antigens and consequently are detected by the enzymatic digestion of substrates. Polyclonal and monoclonal antibodies have been produced against fungal antigens present in culture filtrate, cell fractions, whole cells, cell walls and extracellular components (Narayanasamy 2005).

Species of several seed-borne fungi like *Aspergillus*, *Penicillium* and *Fusarium* have been demonstrated to be potential mycotoxin producers. A monoclonal antibody (MAb) capable of reacting with antigens of 10 field fungi and 27 storage fungi was generated. The presence of fungal pathogens in barley seeds was detected using a polyclonal antibody (PAb) raised against *Penicillium aurantiogriseum* var.

melanoconidium in indirect ELISA test. A clear linear relationship was recorded between absorbance and fungal population increase, suggesting the utility of these antibodies for a broad-spectrum assay to determine the fungal content in seeds (Banks et al. 1993). Rice and corn seeds colonizing fungi, viz. *Aspergillus parasiticus*, *Penicillium citrinum* and *Fusarium oxysporum*, were detected by employing double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test. The absorbance values of ELISA were in good correlation with concentration of mould growth, and the sensitivity of this DAS-ELISA was 1 µg/ml (Chang and Yu 1997).

Karnal bunt disease of wheat caused by *Tilletia indica* is an internationally quarantined fungal disease with a significant impact on international wheat trade as well as quality and quantity of wheat seed. SDS-PAGE analysis suggested that *T. indica* has a protein of 64 kDa weight with antigenic properties. Antibodies specific to this protein specifically reacted with pathogen's teliospores in a microwell sandwich-ELISA and dipstick immunoassay. The detection limit of both of these immunoassays was 1.25 ng/well of purified *T. indica* protein or 40 ng/well of crude spore extract, which distinguished Karnal bunt from all wheat smuts and, to some degree, the rice smut, *T. barclayana* (Kutilek et al. 2001). *Ustilago nuda* causes loose smut in barley and it is an internally seed-borne pathogen. Eibel et al. (2005b) employed a DAS-ELISA test with biotinylated detection antibodies to detect loose smut pathogen in naturally infected barley seeds.

Phomopsis longicolla is a seed-borne fungal pathogen causing *Phomopsis* seed decay of soybean, a major concern for quality seed production in soybean (*Glycine max* L.). This pathogen was detected using indirect ELISA and a modified immunoblot assay, named as seed immunoblot assay (SIBA). The comparative efficiency of both detection assays was evaluated. The problems with nonspecific interference occurred during ELISA test could be solved by employing seed immunoblot assay (SIBA) for detection. In SIBA, infected soybean seeds are transferred to nitrocellulose paper on which the mycelium of *P. longicolla* grows out forming a clearly visible coloured blotch on the nitrocellulose paper after the assay. Since the viable spores can only produce the mycelium, SIBA test is capable of differentiating the living and dead spores of the pathogen, which is a distinct advantage of this technique. In contrast ELISA test results do not offer such vital information (Gleason et al. 1987). Similarly, wheat seeds with different grades of Karnal bunt (*Tilletia indica*) infection could be readily detected by seed immunoblot binding assay (SIBA). After the immuno-processing, coloured imprints were produced on nitrocellulose paper on which infected wheat seeds were placed for vigour test, indicating the presence of viable teliospores of *Tilletia indica* in the wheat seed lots tested (Kumar et al. 1998).

Two methods, viz. PCR-based assay and DAS-ELISA, were developed and evaluated for the detection of *Tilletia caries* (syn. *T. tritici*), a seed-borne fungus causing common bunt in wheat. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed using biotinylated detection antibodies. The presence of bunt pathogen could be detected by PCR in shoots as well as in leaves of infected wheat plants. Except for the closely related *T. controversa*, no cross-reactions with other fungi were observed with both methods. The analysis of results obtained from DAS-ELISA of plant shoots revealed that

artificial inoculation of seeds with *T. caries* at EC 10 was efficient in infecting all host population, with a great variability in the inoculum of pathogen. ELISA employed in the assay was found most suited than PCR assay, allowing precise quantification of the amount of fungal antigen present in the plant (Eibel et al. 2005a). Agar plating method and DAS-ELISA were compared for the detection of *Macrophomina phaseolina*, a seed-borne fungal pathogen and causal agent of root rot diseases in wide host range. The presence of the pathogen was confirmed in four out of five lots by both the detection methods; however, DAS-ELISA format revealed more sensitivity in the detection of the pathogen in higher percentage of seeds as compared to agar plating method which additionally required much time and is inconvenient (Afouda et al. 2009).

Instead of successful detection of some seed-borne fungi, we may conclude that serological techniques have limited values in the detection of seed-borne fungal pathogens, since they contain many nonspecific antibodies which may cause cross-reactions with related and unrelated species concealing the effects of specific antibodies (Dewey 1992; Miller et al. 1992). These assays are widely applied to detect seed-borne viruses, but insufficiency of species-specific antibodies is a major limitation in its wide application for the detection of seed-borne fungal pathogens. Besides, serology-based assays can also detect non-viable fungal propagules, which can lead to imprecise interpretations (Mancini et al. 2016).

5.3.3 Nucleic Acid-Based Detection Methods

Generally, nucleic acid-based techniques resulting in a high level of sensitivity and specificity are used for species-specific detection of seed-borne pathogens. Through these techniques, very small quantities of samples or tissues are sufficient for the detection of pathogens in seeds of various crops. In recent times nucleic acid-based detection methods have become the preferred choice for detection, identification and quantification of seed-borne fungal pathogens. In molecular detection several strategies, viz. polymerase chain reaction (PCR), multiplex PCR, magnetic capture hybridization-PCR, Bio-PCR, loop-mediated isothermal amplification, real-time PCR and DNA barcoding, are available for the detection and identification of pathogens which involves propagation of putative pathogen propagules on a culture medium and subsequent PCR on washes from the culture plates, often using nested PCR primer pairs and sometimes without DNA extraction.

5.3.3.1 Polymerase Chain Reaction (PCR)

Molecular-based methods began after the introduction of PCR in the mid-1980s. Over the past few decades, considerable advancement has taken place in the development of molecular diagnostics for the detection of pathogens in seeds (Molouba et al. 2001). Potential benefits (e.g. rapid, same-day analysis, specific and sensitive tests) this new technology offers, make it extremely attractive. PCR is a method which enables amplification and multiplication, up to a manifold, of the target sequence of DNA. In fungi, internal transcribed spacer (ITS) region has been widely

used to design specific primers to detect the presence of seed-borne infection of fungi. PCR procedures/protocols have been developed for the detection of several seed-borne fungal pathogens associated with seeds of various commercially important crops (Mancini et al. 2016).

Rhynchosporium secalis, a causative agent of barley scald disease, overwinters in the plant debris, and this pathogen is capable of infecting barley seeds without producing conspicuous symptoms, or it may induce typical scald symptoms on seeds. A PCR-based detection method was developed, and in this assay, pathogen-specific primer pairs derived from the ITS region of rDNA of *R. secalis* were effective in detecting this pathogen in the symptomless seed infections. The detection assay revealed the presence of the diagnostic band in the symptomless seeds of susceptible cultivar (Lee et al. 2001). Further, a primer set (RS1 and RS3) derived from the internal transcribed spacer (ITS) regions of ribosomal RNA genes of this pathogen was used to quantify the inoculum of seed-borne infection caused by *Rhynchosporium secalis* in barley using competitive PCR (Lee et al. 2002).

A complex of three species, viz. *Alternaria brassicae*, *A. brassicicola* and *A. japonica*, are responsible for the black spot disease of crucifers. To restrict the transboundary spread of this disease by infected seeds, it is essential to ensure the absence of these pathogenic *Alternaria* species in seed shipments, which constitutes the disease management strategies. A PCR-based diagnostic technique was developed using specific primers developed from sequence analysis of internal transcribed spacer (ITS) regions of nuclear rDNA of *Alternaria brassicae*, *A. brassicicola* and *A. japonica.* This protocol was able to detect these pathogens in DNA extracted from seed macerates (Iacomi-Vasilescu et al. 2002). Another attempt was made to detect *Alternaria brassicae*, an important seed-borne fungal incitant of the black spot disease of crucifers using a polymerase chain reaction (PCR)-based assay. *A. brassicae*-specific primers sets were designed on the basis of the sequences of two clustered genes potentially involved in pathogenicity. The designed two sets of primers were used for conventional and real-time PCR assay. By both the detection methods, *A. brassicae* was specifically detected using DNA extracted from seed (Guillemette et al. 2004).

Rice blast disease, a serious threat to rice production, is caused by *Magnaporthe grisea*. A PCR-based detection protocol was developed using primers designed on the basis of nucleotide sequences of the *mif* 23, an infection-specific gene of *M. grisea*. The primers amplified target DNA from genetically and geographically diverse isolates of *M. grisea*, but not from DNA of other fungi tested, proving the specificity of the primers. The detection limit was ~20 pg of pathogen DNA. This PCR-based seed assay was capable in detecting *M. grisea* in rice seed lots with infestation rates as low as 0.2% (Chadha and Gopalakrishna 2006).

Septoria tritici (teleomorph, *Mycosphaerella graminicola*) is an economically important pathogen causing leaf blotch disease in wheat. *Septoria tritici*, naturally contaminating wheat seeds, was detected employing conventional PCR assay. The species-specific primers developed from strict alignment of ITS and α-tubulin sequences of *Septoria tritici* were used in the diagnostic assay. A single DNA fragment was amplified from DNA of *S. tritici*, but not from DNA of wheat seeds or other fungi selected, the detection limit being 0.5 pg of pathogen DNA (Consolo et al. 2009).

Downy mildew disease caused by biotrophic obligate oomycete *Peronospora arborescens* (Berk.) is one of the most economically important diseases of opium poppy (*Papaver somniferum* L.) worldwide. This pathogen was detected in opium poppy seeds using sensitive nested PCR assay. Two primers designed from the sequences of ITS region of rDNA improved the pathogen detection sensitivity significantly up to 1000-fold compared with single PCR employing same primers. The frequent detection of *P. arborescens* in seeds suggested the likely threat posed by this incitant for rapid spread through the seeds (Montes-Borrego et al. 2009).

The fungus *Corynespora cassiicola* is responsible for target spot disease in soybean in Brazil. This pathogen can be transmitted by seeds and is able to cause severe damage in this crop. Though early diagnostic of the disease by conventional seed testing is possible, species-level detection through these methods is time-consuming and cumbersome. A PCR-based assay was employed using specific GA4-F/GA4-R primers for the detection of *C. cassiicola* in pure culture and in soybean seeds. The pathogen could be detected in infected and inoculated seed samples at the low level of 0.25% (Sousa et al. 2016).

Species-specific detection of *Diaporthe phaseolorum* and *Phomopsis longicolla*, responsible for soybean seed decay, was achieved using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and TaqMan chemistry (Zhang et al. 1999). An ultrasonic processor was used to break the seed coats and cells, enabling the extraction of fungal DNA from soybean seeds. Three TaqMan primer/probe sets were designed, based on DNA sequences of the ITS regions of ribosomal DNA. Primer/probe set PL-5 amplified a 96 bp fragment within the ITS 1 region of *P. longicolla*, *D. phaseolorum* var. *caulivora*, *D. phaseolorum* var. *meridionalis* and *D. phaseolorum* var. *sojae*. An 86 bp DNA fragment was obtained within the ITS 2 region of *P. longicolla* by the set PL-3, whereas set DPC-3 was able to produce 151 bp DNA fragment within the ITS 2 region of *D. phaseolorum* var. *caulivora*. The detection sensitivity of TaqMan primer/probe sets was as low as 0.15 fg (four copies) of plasmid DNA. When using PCR-RFLP for *Diaporthe* and *Phomopsis* detection, the assay was able to detect as little as 100 pg of pure DNA. However, TaqMan detection provided the fastest results of all the methods tested (Zhang et al. 1999).

Tilletia indica, the incitant of Karnal bunt disease, can be correctly identified based on morphological features but the germination of teliospores is time-consuming. A polymerase chain reaction (PCR) detection assay was employed using species-specific primers designed from rDNA-ITS region and 2.3 kb mtDNA fragment of this pathogen. The primer set from ITS region could specifically amplify 570 bp amplicon of *T. indica*, whereas primer set derived from mtDNA was able to amplify 885 bp amplicon of KB pathogen only (Thirumalaisamy et al. 2011). This assay was able to avoid delay in detection and wrong identification of closely related species of *T. indica* from wheat seed lots.

Spot blotch disease of wheat caused by *Bipolaris sorokiniana* is one of the important diseases of wheat. This disease development may take place through seed-borne infections. A quick and reliable PCR-based diagnostic assay was developed to detect *B. sorokiniana* using a pathogen-specific marker derived from genomic DNA. A PCR-amplified DNA amplicon (650 bp) was obtained in *B.*

sorokiniana isolates employing universal rice primer (URP 1F), and it was cloned in pGEMT easy vector and sequenced. A primer pair RABSF1 and RABSR2, of six primers designed based on sequences of PCR-amplified DNA amplicon, amplified a DNA sequence of 600 bp in *B. sorokiniana* isolates. The pathogen could be detected specifically in a mixed population comprising of total 74 isolates of *B. sorokiniana*, *Bipolaris* spp. and other pathogens infecting wheat and other hosts. This single DNA fragment was amplified only from DNA of *B. sorokiniana*, but not from DNA of other species of *Bipolaris* genus and other pathogenic fungi tested, suggesting the specificity of the detection assay, the detection limit being 50 pg of genomic DNA (Aggarwal et al. 2011).

Seed-borne fungal pathogen, *Alternaria radicina*, causes black rot disease of carrot. A PCR-based seed assay was developed for the detection of *A. radicina* from infested carrot seed. PCR primers used in assay were designed based on a cloned random amplified polymorphic DNA (RAPD) fragment of this pathogen. This seed assay was coupled with 5-day incubation under high humidity conditions to increase the fungal biomass. PCR amplification of the target *A. radicina* DNA sequence was improved by the addition of skim milk to the PCR reaction mixture. This PCR-based assay was able to detect the pathogen (*Alternaria radicina*), from seed lots with infestation rates as low as 0.1% (Pryor and Gilbertson 2001).

Pyrenophora graminea, a fungal incitant of leaf stripe disease of barley, is transmitted entirely by seed, and it cannot infect the leaf directly. This pathogen could be detected employing RAPD primers designed using a sequence-characterized amplified region (SCAR) approach. Out of 60 RAPD primers, a set of *P. graminea*-specific primers (PG2 F/R) was obtained that amplified a single DNA fragment (435 bp) from 37 isolates of *P. graminea* tested, but not from other *Pyrenophora* spp. or saprophytes isolated from barley seed. The diagnostic assay was completed within 25 min (including melting point analysis) using a LightCycler, capable of measuring emission of fluorescence from the binding of SYBR Green I dye to the PCR products. The rapidity was coupled with the closed 'in-tube' detection of PCR products which reduces the chances for contamination (Taylor et al. 2001b).

Anthracnose is mainly a seed-borne disease caused by *Colletotrichum lindemuthianum* in bean (*Phaseolus vulgaris*). The pathogen could be detected employing a rapid, specific and sensitive PCR-based detection method. Based on data analysis of sequences of rDNA region consisting of the 5.8S gene and internal transcribed spacers (ITS) 1 and 2 of 4 *C. lindemuthianum* races and 17 *Colletotrichum* spp. downloaded from GenBank, 5 forward primers were designed. One forward primer showing specificity of the detection was selected for use in combination with ITS 4 to specifically detect *C. lindemuthianum*. A 461 bp specific DNA band was obtained from the genomic DNA template of 16 isolates of *C. lindemuthianum*, but not from other *Colletotrichum* species or 10 bean pathogens. A nested PCR protocol was applied to enhance the sensitivity of detection, which enabled the detection of as little as 10 fg of *C. lindemuthianum* genomic DNA and 1% infected seed powder. This detection assay could be accomplished within 24 h against a 2-week period required for culturing the pathogen, and this protocol required no specialized taxonomic expertise (Fig. 5.4) (Chen et al. 2007).

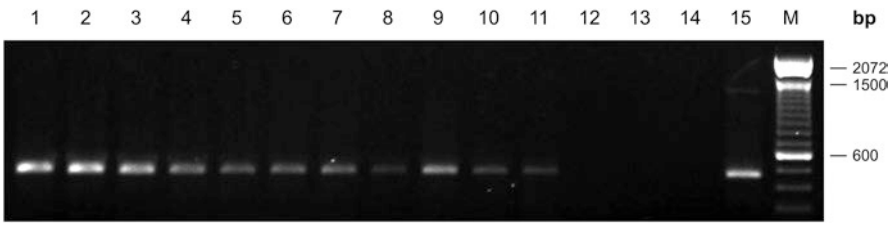

Fig. 5.4 Detection of *Colletotrichum lindemuthianum*-specific DNA fragment in bean seed powder using nested PCR assay. Lanes: M, 100 bp DNA ladder (Invitrogen); 1, 100%; 2, 80%; 3, 60%; 4, 40%; 5, 20%; 6, 10%; 7, 8%; 8, 6%; 9, 4%; 10, 2%; 11, 1%; 12, 0%; 13, anthracnose-resistant bean genotype G2333; 14, negative control (water replaced genomic DNA as the template); 15, positive control (*C. lindemuthianum* DNA). (Courtesy of Chen et al. 2007)

Fusarium wilt of lettuce (*Lactuca sativa*) is a serious threat for the lettuce production around the world. The fungal incitant of the disease *Fusarium oxysporum* f. sp. *lactucae* has the potential for seed-borne spread. *Fusarium oxysporum* f. sp. *lactucae* was detected in the seed of lettuce using a nested polymerase chain reaction (nPCR)-based assay. Sequences of intergenic spacer region of the rDNA were used to design three primers for PCR amplifications. A PCR product (2270 bp) was generated using primer pair GYCF1 and GYCR4C in the first amplification. A 936 bp DNA fragment was amplified employing the forward primer GYCF1 and the nested primer R943 in the second amplification. The nPCR protocol successfully detected the target sequence in genomic DNA of *Fusarium oxysporum* f. sp. *lactucae* at 1 fg/μl. The nPCR seed assay was coupled with a 4-day incubation under high humidity conditions to increase fungal biomass for DNA extraction. In seed lots known amounts of *F. oxysporum* f. sp. *lactucae*-infested seed were mixed with non-infested seed, and this assay detected the pathogen from seed lots with infestation rates as low as 0.1% (Mbofung and Pryor 2010).

5.3.3.2 Multiplex PCR

Multiplex polymerase chain reaction (multiplex PCR) is a valuable molecular tool which offers simultaneous amplification of several DNA amplicons of different sizes, within single PCR reaction (Sint et al. 2012). The multiplex PCR technique was first described by Chamberlain et al. (1988). This technique has various applications and is being commonly used for the identification and detection of pathogens, gene deletion analysis, high-throughput SNP genotyping, linkage and mutation analyses, etc. Since multiplex PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of different sizes that are specific to different DNA sequences, it is capable of detecting several seed-borne pathogenic fungi simultaneously with high sensitivity. Preferably, potential seed health tests can be designed as multiplex assays for particular crops, with their ability to detect all seed-borne pathogens required for phytosanitary purposes.

Multiplex PCR was employed to differentiate members of two groups belonging to *Aspergillus flavus.* The first *Aspergillus flavus* group encloses *A. flavus* and *A. parasiticus* as aflatoxin producers, and the second group includes *A. oryzae* and *A.*

sojae which are best known for their capability to ferment soybean to prepare various food products. Aflatoxigenic strains could be detected, and their differentiation was possible employing the multiplex PCR protocol that minimized the risk of a genotype being a phenotypic producer of aflatoxin. This assay was based on four genes involved in aflatoxin biosynthesis, viz. norsolorinic acid reductase (nor-1), versicolorin A dehydrogenase (ver-1), sterigmatocystin O-methyltransferase (omt-1) and a regulatory protein (apa-2) (Chen et al. 2002). Multiplex PCR could successfully detect *Fusarium* species within *Fusarium* head scab complex (Waalwijk et al. 2003) and *Rhynchosporium secalis*, a seed-borne fungal incitant causing economically important leaf blotch disease of barley (Fountaine et al. 2007). There may be chances that a seed may harbour very closely related species of fungi. The occurrence of very closely related fungal species on the seed may lead to overlapping while simultaneous detection. In multiplex PCR, possible problem of overlapping of amplicons with similar sizes could be overcome using primers, already dyed with different colour fluorescent dyes (Sint et al. 2012).

Comparison of two PCR-based protocols was done for the detection of *Fusarium verticillioides* and *Fusarium subglutinans*, important fungal pathogens of maize and other cereals worldwide. PCR-based protocols were used for the identification of these pathogens targeting the *gaoB* gene, which codes for galactose oxidase. The designed primers recognized isolates of *F. verticillioides* and *F. subglutinans* obtained from maize seeds from several regions of Brazil but did not recognize other *Fusarium* spp. or other fungal genera. A multiplex PCR diagnostic protocol was capable to simultaneously detect the genomic DNA from *F. verticillioides* and *F. subglutinans* growing in artificially or naturally infected maize seeds (Faria et al. 2012).

Fusarium culmorum causes disease complex, viz. seed rot, seedling blight and ear rot in maize. The multiplex PCR assay was standardized targeting trichothecene metabolic pathway genes, viz. *Tri6*, *Tri7* and *Tri13*, for the detection of trichothecene (DON/NIV) chemotypes and rDNA gene for the specific detection of *Fusarium culmorum* species in freshly harvested maize seeds. The analysis of primers employed in multiplex PCR assay revealed that 94 isolates were able to produce deoxynivalenol/nivalenol DON/NIV. The practical usefulness of mPCR assay was validated by comparing these results with high-performance thin-layer chromatography (HPTLC) and found that mPCR results equivocally matched with the HPTLC chemical analysis for field samples (Venkataramana et al. 2013).

5.3.3.3 Magnetic Capture Hybridization (MCH)-PCR

Interference caused by inhibitory compounds of seed extracts in conventional PCR is a major limitation affecting both assay sensitivity and reliability. The magnetic capture hybridization-PCR (MCH-PCR) has the advantage that the MCH process purifies and concentrates the DNA of interest while removing non-target DNA and other substances that can inhibit the in vitro enzymatic manipulation of nucleic acids that are normally found in complex sharing biological material. In MCH-PCR, magnetic beads coated with single-stranded DNA probes are used to capture DNA fragments which are further used for PCR amplification. This technique has been successfully used to detect fungi, viruses and bacteria in materials containing

PCR inhibitory compounds (Jacobsen 1995). With the use of MCH-PCR, the detection sensitivity can be enhanced up to 10- to 100-fold as compared to conventional PCR protocol.

Complex of three species of *Botrytis*, viz. *B. aclada*, *B. allii* and *B. byssoidea*, are responsible for neck rot disease in onion. The pathogens of this disease are transmitted by onion seeds. An MCH-PCR diagnostic protocol was employed for the rapid and sensitive detection of *B. aclada* in onion seed samples. The DNA of *B. aclada* could be detected using MCH-PCR diagnostic protocol, in aqueous solutions prepared from seed extract with detection limit as 100 fg of fungal DNA/ml. MCH-PCR protocol was more sensitive and efficient than normal PCR, in detecting the fungus *B. aclada* in seed lots with 4.8% and 9.9% infection in naturally infested seeds. MCH-PCR detection assay could be completed within 24 hours against a 10–14-days period required in conventional methods to test onion seeds (Walcott et al. 2004). Two important seed-borne pathogens of cucurbits could be detected by employing a magnetic capture hybridization (MCH) multiplex real-time PCR assay. This assay offered the improved simultaneous detection of two different pathogens in cucurbit seed lots, viz. *Acidovorax avenae* subsp. *citrulli*, a causal agent of bacterial fruit blotch, and *Didymella bryoniae*, a fungal incitant of gummy stem blight disease (Ha et al. 2009).

5.3.3.4 Bio-PCR

Bio-PCR enables the enhancement of fungal biomass since seed-borne fungi generally infect the host seeds at very low concentration of inoculum, and therefore the DNA of the fungal pathogen is not enough for the subsequent reactions, limiting the use of conventional PCR-based detection. This technique was developed by Schaad et al. (1995) primarily to detect a seed-borne bacterium from bean seed extracts. Later, this technique was also proved to be efficient for detection of fungi (Munkvold 2009). Bio-PCR is mainly applied for fungi and bacteria. In this detection method, a pre-assay incubation step is coupled with PCR process. Bio-PCR consists of the preventive growth of non-target pathogens on selective medium and selective increase in the biomass of target microorganisms, followed by DNA extraction and amplification by PCR (Schaad et al. 1995).

Bio-PCR has been proven successful in the detection/identification of *Tilletia indica* teliospores in wheat seed samples (Schaad et al. 1997). In Bio-PCR, standard deep-freeze blotter method was utilized as pre-assay incubation to increase the fungal biomass of *Alternaria dauci* and *A. radicina* from infected seeds of carrot and was detected using of specific primers of *A. dauci* and *A. radicina* during the PCR assay (Konstantinova et al. 2002).

Though Bio-PCR offers several advantages over conventional PCR assay, like it is highly sensitive, eliminates PCR inhibitors and avoids false positives due to dead cells since it detects live cells only (Marcinkowska 2002), there are some limitations of Bio-PCR also, viz. if selective media are used, the method becomes more expensive and pre-assay requires 5–7-days period to increase fungal growth, which substantially increases the time required for the completion of the assays (Mancini et al. 2016).

5.3.3.5 Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is one of the novel nucleic acid amplification technologies that enables the synthesis of large amounts of DNA in a short period of time with high specificity. This technique was developed by Notomi et al. (2000) as a simple, cost-effective and rapid method for the specific detection of genomic DNA (Mancini et al. 2016). In the future it may be a potential alternative to PCR, since LAMP protocol does not require thermocycler apparatus. LAMP uses a pair of four or six oligonucleotide primers with eight binding sites hybridizing specifically to diverse areas of a target gene and a thermophilic DNA polymerase from *Geobacillus stearothermophilus* for DNA amplification. Additionally, being a highly specific diagnostic protocol, the amplification efficiency of LAMP is extremely high, which provides improved sensitivity, and can overcome the problem of inhibitors that usually adversely affect PCR methods (Fu et al. 2011). LAMP products can be visualized by gel electrophoresis, using magnesium pyrophosphate, which enhances precipitation of amplified DNA (Fukuta et al. 2003; Nie 2005), with a real-time turbidity reader (Fukuta et al. 2004; Mori et al. 2004), or with the addition of an intercalating dye, such as SYBR Green I, which produces a colour change in the presence of target phytopathogen (Iwamoto et al. 2003; Mumford et al. 2006).

Fusarium graminearum is the major causative agent among the species complex of *Fusarium* head blight of small cereals and is potential producer of the mycotoxins, viz. deoxynivalenol, nivalenol and zearalenone. The pathogen could be detected by employing LAMP assay, based on the *gaoA* gene (galactose oxidase) of *Fusarium graminearum*. Amplification of DNA during the reaction was indirectly detected in situ by using calcein fluorescence as a marker, circumventing the use of time-requiring electrophoretic analysis. The LAMP protocol was able to detect the presence ~2 pg of purified target DNA per reaction within 30 min, specifically from DNA of *F. graminearum* (Niessen and Vogel 2010).

Fusarium oxysporum f. sp. *ciceris* (Foc), the incitant of *Fusarium* wilt, is both a soil-borne and seed-borne fungus. It is one of the most devastating pathogens of chickpea, causing major economic losses ranging from 10% to 40% worldwide. It is estimated to cause a 10–15% yield loss annually in India (Haware and Nene 1982). A loop-mediated isothermal amplification (LAMP) assay was developed targeting the elongation factor 1 alpha gene sequence for visual detection of Foc. The LAMP reaction was optimal at 63 °C for 60 min. In the presence of hydroxynaphthol blue (HNB) added before amplification, DNA of Foc developed a characteristic sky blue colour, whereas this colour was absent in the DNA of six other plant pathogenic fungi. Later, gel electrophoresis analysis confirmed the results obtained with LAMP and HNB. The detection limit of this LAMP assay for Foc was 10 fg of genomic DNA per reaction, against 100 pg of conventional PCR (Ghosh et al. 2015).

Karnal bunt in wheat caused by a fungus *Tilletia indica* is a quarantine disease, and therefore timely and specific detection of the pathogen is very essential. The pathogen could be detected specifically with rapidity employing the loop-mediated isothermal amplification (LAMP) at 62 °C. Four major unique regions were identified in *T. indica* through analysis of alignment of the mitochondrial DNA of *T. indica*

and *T. walkeri.* Six LAMP primers designed from one of these major unique regions in *T. indica* could amplify *T. indica* DNA. Among 17 isolates of *T. indica*, *T. walkeri*, *T. horrida*, *T. ehrhartae* and *T. caries*, this protocol offered highly specific detection of *T. indica.* Endpoint detection with the naked eye could be possible using the fluorescent chemical calcein. The diagnostic assay could be completed in 30 min, offering similar sensitivity as with conventional PCR. The specificity issues that occurred during PCR-based detection protocols due to the high DNA homology of *T. indica* with other *Tilletia* species, especially *T. walkeri*, could be solved by employing this technique for detection (Gao et al. 2016).

Phomopsis longicolla is an important seed-borne fungal pathogen responsible for the deterioration in the seed quality of soybean. The pathogen could be detected employing loop-mediated isothermal amplification (LAMP) diagnostic assay based on transcription elongation factor 1-α (*TEF1-α*), identified as a suitable target for the detection of *P. longicolla.* This LAMP diagnostic assay, with great specificity, was capable to detect all 54 isolates of *P. longicolla* from the rest of the 41 isolates of other fungi tested. Before the amplification of LAMP products, hydroxynaphthol blue (HNB) was added, and a sky blue colour was only developed in the presence of *P. longicolla*, while other fungal isolates failed to show colour change. The detection limit of the assay was 100 pg/μL fungal DNA, and additionally the assay also detected *P. longicolla* from diseased soybean tissues and residues from different origins (Dai et al. 2016).

Anthracnose is a worldwide occurring fungal disease of soybean. This disease is primarily caused by *Colletotrichum truncatum.* Rapid and direct detection of the pathogen in diseased soybean tissues could be possible employing a loop-mediated isothermal amplification (LAMP) assay. A pair of species-specific primers was designed using the target gene *Rpb1* (that codes for the large subunit of RNA polymerase II). During the screening, species-specific primers amplified the genomic DNA of *Colletotrichum truncatum* at 62 °C over 70 min. The presence of *C. truncatum* could be confirmed by a yellow-green colour (visible to the unaided eye), developed in LAMP reaction products after addition of SYBR Green I dye. This *Rpb1*-Ct-LAMP assay could successfully diagnose soybean anthracnose in field samples collected from various locations of China and was able to detect *C. truncatum* in soybean seeds from farmers' markets, the detection limit being 100 pg (per μL genomic DNA of pathogen) (Tian et al. 2017).

5.3.3.6 Real-Time PCR

It is a laboratory technique of molecular biology based on PCR, which consists of amplification and simultaneous detection or quantification of targeted DNA molecule. Real-time PCR consists of coupling DNA amplification with fluorescent substances which can be easily measured, giving an indirect measurement of DNA amplification. This is the case of TaqMan (Heid et al. 1996; Taylor et al. 2001a) where fluorescence is directly linked to the excision of reporter dye molecules, which is directly related to DNA amplification. In contrast to other detection techniques, a much quicker and more sensitive, quantitative assay could be provided by real-time PCR assays.

Detection through real-time PCR was reported for *Didymella bryoniae*, an incitant of gummy stem blight of cucurbits (Ling et al. 2010). A real-time fluorescent polymerase chain reaction (PCR) assay was developed using SYBR Green chemistry to quantify three species of *Botrytis*, viz. *B. aclada*, *B. allii* and *B. byssoidea*, associated with onion (*Allium cepa*) seed that are also able to induce neck rot of onion bulbs (Chilvers et al. 2007). The nuclear ribosomal intergenic spacer (IGS) regions of target and non-target *Botrytis* spp. were sequenced and aligned and used to design a primer pair specific to *B. aclada*, *B. allii* and *B. byssoidea.* The primers reliably detected 10 fg of genomic DNA per PCR reaction extracted from pure cultures of *B. aclada* and *B. allii* (Chilvers et al. 2007).

Simultaneous detection of *Pantoea ananatis* and *Botrytis allii* was performed in onion seeds using magnetic capture hybridization and real-time PCR (Ha and Walcott 2008). Montes-Borrego et al. (2011) have achieved real-time PCR quantification of *Peronospora arborescens*, the opium poppy downy mildew pathogen, in seed stocks and symptomless infected plants. Ioos et al. (2012) have used duplex real-time PCR tool for sensitive detection of the quarantine oomycete *Plasmopara halstedii* in sunflower seeds. A real-time PCR assay utilizing SYBR Green was developed to detect *V. dahliae* associated with spinach seed (Duressa et al. 2012). More recently, a multiplex TaqMan real-time PCR assay was developed for the detection of spinach seed-borne pathogens, viz. *Peronospora farinosa* f. sp. *spinaciae*, *Stemphylium botryosum*, *Verticillium dahliae* and *Cladosporium variabile*, that cause economically important diseases on spinach (Feng et al. 2014). They tested TaqMan assays on DNA extracted from numerous isolates of the four target pathogens, as well as a wide range of non-target, related fungi or oomycetes and numerous saprophytes commonly found on spinach seed. Multiplex real-time PCR assays were evaluated by detecting two or three target pathogens simultaneously. Singular and multiplex real-time PCR assays were also applied to DNA extracted from bulked seed and single spinach seed (Feng et al. 2014). *Fusarium oxysporum* f. sp. *phaseoli* is a devastating pathogen that can cause significant economic losses and can be introduced into fields through infested common bean (*Phaseolus vulgaris*) seeds. Robust seed health testing methods can be helpful in preventing long-distance dissemination of this pathogen by contaminated seeds. A rapid real-time PCR assay (qPCR) protocol was developed for the detection and quantification of *Fusarium oxysporum* f. sp. *phaseoli* in common bean seeds. SYBR Green and TaqMan qPCR methods were compared directly using primers based on the Fop virulence factor *ftf1*. Both qPCR assays detected infection in seed at low levels (0.25%); however, the TaqMan assay was found more reliable at quantification than the SYBR Green assay (Sousa et al. 2015). To ensure adequate specificity and sensitivity and comparable amplification efficiency of different pathogens in real-time PCR assays, it is critical to choose the appropriate target DNA fragments to design the primers and probes (Mancini et al. 2016). Recently, a real-time PCR-based marker was developed for the detection of teliospores of *Tilletia indica* in soil (Gurjar et al. 2017).

5.3.3.7 DNA Barcoding

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species (Hebert et al. 2003). The nuclear ribosomal internal transcribed spacer (ITS) region is a recently proposed DNA barcode marker for fungi (Schoch et al. 2012). Identification of universal barcoding regions is important to detect seed-borne fungi. Internal transcribed spacer (ITS) has been used as the primary barcode marker for fungi based on its ability to successfully identify inter- and intraspecific variation among a wide range of fungi. An ideal barcoding gene should be sufficiently conserved to be amplified with wide range of primers, however divergent enough to identify closely related species. Other applications include, for example, identifying plant leaves even when flowers or fruits are not available and identifying insect larvae (which may have fewer diagnostic characters than adults and are frequently not well-known) (Kress et al. 2005). Barcoding regions of some important seed-borne fungi are given in Table 5.3.

5.3.4 Next-Generation Sequencing (NGS)

After its first application in basic biological research, NGS technologies have been extended to other fields of application, which have included plant disease diagnosis. As NGS has been a valuable technique for the rapid identification of disease-causing agents from infected plants, it can also be applied to the detection of fungal pathogens in seeds. This technique has been applied to study the mycobiome of wheat seed, using 454 pyrosequencing, allowing the identification of several fungal genera (Nicolaisen et al. 2014). In view of this technology's great potential, the major sequencing platforms used for genome and other sequencing applications, 454 sequencing, AB/SOLiD technology and Illumina/Solexa sequencing are described below.

The first NGS technology that was proposed by Roche for the market was 454 sequencing, which bypasses cloning steps by taking advantage of PCR emulsion, a highly efficient in vitro DNA amplification method. It is based on colony sequencing and pyrosequencing. The pyrosequencing approach is a sequencing-by-synthesis technique that measures the release of pyrophosphate by producing light, due to the cleavage of oxyluciferin by luciferase. Currently, the 454 platform can produce 80–120 Mb of sequence in 200 to 300 bp reads in a 4 h run (Morozova and Marra 2008; Barba et al. 2014).

AB/SOLiD technology is sequencing by oligonucleotide ligation and detection (SOLiD). It depends on ligation-based chemistry with di-base labelled probes and uses minimal starting material. Sequences are obtained by measuring serial ligation of an oligonucleotide to the sequencing primer by a DNA ligase enzyme. Each SOLiD run requires 5 days and generates 3–4 Gb of sequence data with an average read length of 25–35 bp (Mardis 2008; Morozova and Marra 2008).

Illumina/Solexa sequencing is similar to the Sanger-based methods, because it uses terminator nucleotides incorporated by a DNA polymerase. However, Solexa

Table 5.3 Barcoding regions of some seed-borne fungi

S. no.	Barcoding regions	Seed-borne fungi	References
1.	Translation elongation factor-1 alpha (TEF-1 alpha)	*Fusarium* spp.	Amatulli et al. (2010)
2.	Intergenic spacer sequence (IGS)	*Fusarium verticillioides*	González-Jaen et al. (2004)
3.	Internal transcribed spacers (ITS) region	*Alternaria alternata*, *A. infectoria* and *A. triticina*	Links et al. (2014)
		Peronospora arborescens	Landa et al. (2007)
		Albugo candida	Robideau et al. (2011)
		Pseudoperonospora cubensis	Robideau et al. (2011)
4.	Lpv gene	*Phytophthora cinnamoni*	Kong et al. (2003)
5.	*Cytochrome* C oxidase 1	*Aspergillus* spp.	Geiser et al. (2007)
		Albugo candida	Robideau et al. (2011)
		Penicillium spp.	Seifert et al. (2007)
		Pseudoperonospora cubensis	Robideau et al. (2011)
6.	Pot2 transposon	*Magnaporthe oryzae*	Kachroo et al. (1994)
7.	LSU regions	*Albugo candida*	Robideau et al. (2011)
		Pseudoperonospora cubensis	Robideau et al. (2011)
8.	α-Tubulin sequences	*Septoria tritici* and *Rhynchosporium secalis*	Rohel et al. (1998)
9.	NADH dehydrogenase	*Fusarium* sp.	Kamil et al. (2015)

terminators are reversible, allowing continuation of polymerization after fluorophore detection and deactivation. Sheared DNA fragments are immobilized on a solid surface (flow-cell channel), and solid-phase amplification is performed. At the end of the sequencing run (4 days), the sequence of each cluster is computed and subjected to quality filtering to eliminate low-quality reads. A typical run yields about 40–50 Mb (typical read length of 50–300 bp; Varshney et al. 2009; El-Metwally et al. 2014).

The availability of these NGS assays means that they should now be used to examine the presence of pathogens on or in seeds, especially 454 sequencing that has already been proven to identify fungi on seeds; they may be proved useful in the future for routine seed diagnosis.

5.3.5 Other Newly Developed Diagnostic Techniques

5.3.5.1 Biospeckle Laser Technique

A recently applied tool that can reveal the presence of pathogenic fungi on seeds is known as the 'biospeckle' laser technique. This technique is based on the optical phenomenon of interference that is generated by a laser light that interacts with the seed coat. Examination of seeds under laser light allows the identification of areas with different activities (Braga et al. 2005; Rabelo et al. 2011). As fungi present on the seeds have biological activity, this method can detect their presence on seeds.

5.3.5.2 Videometer Lab Instrument

One more recently developed tool called videometer lab instrument that can distinguish infected seeds from healthy seeds is a multispectral vision system also useful to determine the colour, texture and chemical composition of seed surfaces (Boelt et al. 2018). The combinations of the features from images captured by visible light wavelengths and near-infrared wavelengths were worthwhile in the separation of healthy spinach seeds from seeds infected by *Stemphylium botryosum*, *Cladosporium* spp., *Fusarium* spp., *Verticillium* spp. or *A. alternata* (Olesen et al. 2011). Seed quality of castor (*Ricinus communis* L.) based on seed coat colour was predicted employing multispectral imaging technology using VideometerLab instrument. This technology was able to distinguish viable seeds from dead seeds with 92% accuracy, suggesting its utility for seed deterioration caused by fungal pathogens (Olesen et al. 2015).

5.4 Summary

Seed health has become an important quarantine issue, mainly in the international movement of seeds and germplasm exchange. Thus, it is essential to make sure that no potentially damaging pathogens are established on seeds. Conventional seed detection methods including visual examination, selective media, seedling grow-out assay and the serological assays have been used extensively, but all have limitations like inefficiency and sensitivity. The molecular methods have shown great potential for improving pathogen detection in seeds as it embodies many of the key characteristics including specificity, sensitivity, rapidity, ease of implementation, interpretation and applicability. PCR and its modifications including Bio-PCR and MCH-PCR may offer opportunities to evade inhibitory compounds while improving detection of seed-borne pathogens. Further, reduced cost and more efficiency will ultimately allow DNA-based detection methods to replace the vast range of seed detection assays presently engaged and will provide advanced detection abilities essential for healthy seedling establishment. A comparative analysis of various seed detection methods based on the time required for completion, sensitivity, ease of application and specificity along with the examples of fungi detected on seeds using the particular technique has been summarized below in Table 5.4.

Table 5.4 General features of seed detection assays including the time required for completion, sensitivity, ease of application, specificity and applicability for the detection of fungi on seed (Walcott 2003; Mancini et al. 2016)

Type of assay	Time required	Sensitivity	Ease of application	Specificity	Ease of implementation	Examples
Visual examination	5–10 min	Low	Simple and inexpensive (requires experience)	Low	Mycological skills required	*Phomopsis* spp., *Cercospora kikuchii*, *Peronospora manshurica*/soybean seed; *Cylindrocladium parasiticum*/peanut seed; *Colletotrichum dematium*/chilli seed; *Septoria apii*/celery seed
Seed washing technique	10–30 min	Low	Simple and inexpensive	Low	Mycological skills required	*Peronospora manshurica*/soybean seed
Semi-selective media	2–14 days	Moderate	Simple and inexpensive	Low–moderate	Mycological skills required	*Alternaria brassicicola* (crucifer seeds)
Seedling grow-out assay	2–3 weeks	Low	Simple, inexpensive and robust	Low	Mycological skills required	Fungal seedling diseases caused by *Alternaria*, *Bipolaris*, *Fusarium*, *Pyricularia*, etc.
Freeze blotter incubation	1 week	Low/moderate	Simple and inexpensive	Moderate	Mycological skills required	*Alternaria dauci*, *Alternaria radicina*/carrot seed; *Leptosphaeria maculans*/Brassicaceae seed
Agar medium incubation	5–7 days	Low/moderate	Simple and inexpensive	Moderate	Mycological skills required	*Alternaria dauci*, *Alternaria radicina*, *Alternaria carotiincultae*/carrot seed; *Verticillium dahliae*, *Fusarium* spp./Cucurbitaceae seed; *Botrytis* spp./onion seed
Serology-based assay	2–4 h	Moderate–high	Simple, moderately expensive and robust	Moderate–high	Ease of interpretation	*Macrophomina phaseolina*/cowpea seed

(continued)

Table 5.4 (continued)

Type of assay	Time required	Sensitivity	Ease of application	Specificity	Ease of implementation	Examples
Conventional DNA extraction and PCR	5–6 h	High	Complicated; easy to interpret, expensive	Very high	Molecular biology skills required, ease of interpretation	*Alternaria brassicae*, *Leptosphaeria maculans*/Brassicaceae seed; *Ascochyta lentis*/lentil seed; *Alternaria radicina*/carrot seed; *Phoma valerianella*/lamb's lettuce seed; *Fusarium oxysporum* f. sp. *basilici*/basil seed
Bio-PCR (selective target colony enrichment followed by PCR)	5–7 days	Very high	Complicated, expensive	Very high	Molecular biology skills required, ease of interpretation	*Alternaria dauci*, *Alternaria radicina*/carrot seed; *Alternaria brassicae*, *Leptosphaeria maculans*/Brassicaceae seed; *Ascochyta rabiei*/chickpea seed; *Fusarium oxysporum* f. sp. *lactucae*/ lettuce seed
MCH-PCR (magnetic capture hybridization and PCR)	2–5 h	Very high	Complicated, expensive	Very high	Molecular biology skills required	*Didymella bryoniae*/Cucurbitaceae seed; *Botrytis* spp./onion seed
Nested PCR	5–6 h	Very high	Complicated, expensive	High	Molecular biology skills required, ease of interpretation	*Colletotrichum lindemuthianum*/bean seeds; *Fusarium oxysporum* f. sp. *lactucae*/lettuce seeds
Real-time PCR	40–60 min	Very high	Complicated, expensive	Very high	Molecular biology skills required	*Alternaria brassicae*, *Plasmodiophora brassicae*/Brassicaceae seed; *Didymella bryoniae*/Cucurbitaceae seed; *Botrytis* spp./onion seed; *Verticillium dahliae*/spinach seed; *Colletotrichum lindemuthianum*/bean seed; *Fusarium oxysporum* f. sp. *basilici*/basil seed
DNA microarrays	6 hours	Very high	Complicated, expensive	Very high	Molecular biology skills required	*Botrytis cinerea*, *B. squamosa* and *Didymella bryoniae*
Laser biospeckle technique	High	High	Complicated, expensive	High	Technological skills required	*Fusarium oxysporum*, *Aspergillus flavus*, *Sclerotinia* spp./bean seed
Videometer	High	High	Complicated, expensive	High	Technological skills required	*Stemphylium botryosum*, *Cladosporium* spp., *Fusarium* spp., *Verticillium* spp., *Alternaria alternata*/spinach seed

5.5 Challenges and Future Directions

Besides the unique advantages offered by the various seed/plant disease detection methods, each method has its own limitations. Before adopting these assays, it is critical to rigorously evaluate their applicability, precision and accuracy in real-world, high-throughput testing of naturally infested seeds. To ensure that these assays work, they must be validated in stringent multilaboratory tests which evaluate their reproducibility and repeatability. Only assays evaluated in this manner should be considered for testing of commercial seeds.

5.6 Conclusion

In this chapter, we reviewed the currently existing methods for detection of seed-borne fungal phytopathogens. Although the conventional methods are widely used for the detection of seed-borne fungal phytopathogen presently, they are relatively difficult to operate, require expert technicians and are time-consuming for data analysis. Ultimately, improved protocols based upon PCR, ELISA, etc. will be available for the detection of all seed-borne pathogens and may supersede conventional detection methods.

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6 Detection of Seed and Propagating Material-Borne Bacterial Diseases of Economically Important Crops

Dinesh Singh and Priyanka Singh Rathaur

Abstract

Seeds and propagating materials of plants are the primary source of pathogen inoculum to cause diseases. These materials also transmit the pathogen from one place to other places and establish the disease in a new area. Hence, it is an utmost requirement to detect the pathogen of a particular crop before transportation and sowing to ensure that no potentially damaging pathogens are introduced in the field through seeds and planting materials. This can be most effectively accomplished by keeping out pathogens from seed lots by either discarding or treating seeds with chemicals. Various conventional methods for the detection of pathogens such as visual examination, selective growth media, serological methods, and bioassay have been used commonly. But these methods have disadvantages like inefficiency, less specificity, less sensitivity, and more time-consuming. Now-a-days, polymerase chain reaction (PCR) has more potential to improve bacterial pathogen detection in seeds as well as planting materials. There are advanced techniques like BIO-PCR, immunomagnetic separation-PCR (IMS-PCR), and magnetic capture hybridization-PCR (MCH-PCR) which reduce inhibitory compounds during PCR, which further improve the detection level of bacterial pathogens from seeds and planting materials. IMS-PCR and MCH-PCR are more attractive due to their simple and universally applicable methods to test seeds for different culturable and non-culturable bacterial pathogens. However, it is difficult to adapt their applicability for routine testing of seed under the laboratory. It should be ensured that these methods should work and these methods must be validated in multi-laboratory tests thoroughly and these tests should be reproducible and repeatable before their commercialization.

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6.1 Introduction

Bacteria are the second important pathogens after fungi which cause important plant diseases. In India, more than 200 bacterial diseases of plants are known, and out of these, atleast 75 diseases are of great economic importance. Throughout the history of agriculture, seed-borne diseases have been one of the most serious problems in crops' cultivation. Seed is the most important input in crop production by gaining 50% in productivity which is the main reason to use improved seeds, and it increases alone productivity of crops with the range of 10–15%. Seed-borne disease is defined as a particular plant disease that is transmitted by seed. However, the transmission of pathogen on seed is sometimes insignificant as compared to the pathogen population that present either in soil or on weed plants. But in other cases, the pathogen transmission through seeds is the main means by which a disease spreads. They damage the crops by reduction of assimilating surface due to yellowing and necrosis, death of its parts or complete plants, malformation, and/or growth reduction. Losses caused by bacterial diseases are mainly economic, but may also be personal or aesthetic. The estimated loss due to seed-borne pathogens varies from disease to disease, from environmental conditions, and also from place to place. However, the losses due to some important seed-borne bacterial diseases caused by *Xanthomonas oryzae* pv. *oryzae*, *Pseudomonas syringae* pv. *glycinea*, and *X. campestris* pv. *campestris* were significant, 10, 64, and 5% in crops as rice, soybean, and cole crops, respectively. To obtain better and high-quality seed, many factors such as seed viability, seed health, and genetic purity should be taken into account in production. A "good-quality seed" should have maximum germination percentage and genetic and physical purity (the seed should be uniform in shape, size, and color and free from inert matter), and seed health should be ensured which means it should be free from diseases and pests. The seed should be free from seed-borne pathogens which are the primary means of checking the entry of pathogens, particularly new pathogens, into a new area. While sowing of the infected seeds may cause widespread distribution of disease within the crop and assent to increase number of initial infection points, from which the disease may spread (Table 6.1). It was noticed that majority of farmers use their own saved seeds from the previous harvest to raise crop in the next season, which are uncertified seed. Sometimes, they borrow the seeds from neighbors or purchase from local markets, which are the factors that embolden spread and entry of new diseases. Infested seeds play an important role in the reemergence of the diseases, pathogen movement across international borders, or the entry of the diseases into new areas. A lot of work has been done to improve the sensitivity and selectivity to assay the seed-borne pathogen from seeds by using advanced techniques like flow cytometry and polymerase chain reaction. This chapter focuses on the importance, detection, and diagnosis challenges of seed-borne bacterial diseases.

Table 6.1 Seed-borne bacterial diseases and their causal agents

Crop	Pathogens/disease
Wheat	*Pseudomonas syringae* pv. *syringae* (blight), *Xanthomonas campestris* pv. *translucens* (bacterial leaf streak and black chaff)
Maize	*Pantoea stewartii* subsp. *stewartii* (Stewart's diseases and wilt), *Clavibacter michiganensis* subsp. *nebraskensis* (bacterial wilt and blight)
Rice	*X. oryzae* pv. *oryzae* (bacterial leaf blight), *X. oryzae* pv. *oryzicola* (bacterial leaf streak), *Acidovorax avenae* pv. *avenae* (bacterial blight and leaf stripe), *Burkholderia glumae* (bacterial grain rot)
Oat	*Acidovorax avenae* pv. *avenae* (bacterial blight and leaf stripe)
Bean	*P. syringae* pv. *phaseolicola* (halo blight), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (bacterial wilt), *X. axonopodis* pv. *phaseoli* (common blight), and *X. fuscans* var. *fuscans* (fuscans blight)
Soybean	*P. syringae* pv. *glycinea* (bacterial blight)
Chickpea	*Rhodococcus fascians* (leafy gall)
Cereal grasses	*Rathayibacter* sp. (yellow slime)
Alfalfa	*C. michiganensis* subsp. *insidiosus* (bacterial wilt)
Tomato	*P. syringae* pv. *syringae*, *P. syringae* pv. *tomato* (bacterial speck), *Xanthomonas* spp. (bacterial leaf spot), *C. michiganensis* subsp. *michiganensis* (bacterial canker)
Carrot	*X. campestris* pv. *carotae* (bacterial blight)
Onion	*P. ananatis* (center rot), *B. cepacia* (sour skin rot)
Crucifers	*X. campestris* pv. *campestris* (black rot), *P. cannabina* pv. *alisalensis* (bacterial blight), *Pseudomonas syringae* pv. *maculicola* (bacterial leaf spot)
Cucurbits	*P. syringae* pv. *lachrymans* (angular leaf spot)
Lettuce	*X. campestris* pv. *vitians* (bacterial leaf spot)
Potato	*Ralstonia solanacearum* (brown rot and wilt), *Erwinia carotovora* subsp. *carotovora* (soft rot), *E. carotovora* subsp. *atroseptica* (soft rot and black leg)
Pea	*P. syringae* pv. *pisi* (bacterial blight)
Pepper	*X. euvesicatoria*, *X. vesicatoria* (bacterial leaf spot)
Cotton	*X. citri* pv. *malvacearum* (black arm and angular leaf spot)
Cluster bean	*X. campestris* pv. *cyampsidis* (bacterial blight)
Sesame	*X. campestris* pv. *sesami* (bacterial blight)
Watermelon	*Acidovorax avenae* pv. *citrulli* (bacterial fruit blotch)
Propagating material	
Pomegranate	*X. axonopodis* pv. *punicae* (bacterial blight)
Orange	*Xylella fastidiosa* (citrus variegated chlorosis), *Candidatus* Liberibacter asiaticus (citrus greening)
Sugarcane	*Leifsonia xyli* subsp. *xyli* (ratoon stunting)
Stone fruits	*Xanthomonas arboricola* pv. *pruni* (blight)
Geraniums	*Ralstonia solanacearum* (wilt), *Xanthomonas pelargonii* (bacterial blight)

6.2 Major Seed-Borne Bacterial Diseases

6.2.1 Black Rot Disease of Crucifers

Black rot disease of crucifers caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson is a most serious seed-borne disease, which significantly reduces the yield of crops (>50%) all over the world under favorable environmental conditions (Williams 1980). Black rot is a vascular disease in which pathogenic bacteria invade the xylem, colonize the mesophyll, and ultimately spread throughout the plant. *X. campestris* pv. *campestris* survives in seeds, soil, and, especially, plant residues and weeds of Cruciferae family near the field, for a longer period. These sources of pathogen inoculum cause recurrence of the black rot disease in the nursery as well as in the main field of crucifer crops annually. To prevent black rot disease, it is a primary requirement to test the seeds and ensure that the seed is pathogen-free before sending it to growers for sowing. Losses incurred through the use of infected seeds or seedlings can be avoided through the use of efficient detection methods.

6.2.2 Bacterial Canker of Tomato

Bacterial canker caused by *Clavibacter michiganensis* pv. *michiganensis* is seed-borne and usually occurs sporadically in the field. The pathogenic bacteria can survive for short duration in soil, greenhouse, and equipment, whereas for longer periods in plant residues. The disease is a vascular (systemic) and parenchymatal (superficial) disease, producing a wide range of symptoms on the plants resulting in the loss of photosynthetic area, wilting and premature death, as well as reduced market value of fruit. It is a very destructive disease in nature; hence, vigilance must be done when going for the selection and handling of seed stocks, preparation and management of greenhouse soil beds or bags, and selection and preparation of ground for field production. Early detection of the disease, especially in greenhouse crops, is necessary for the successful control of canker disease.

6.2.3 Bacterial Blights

Bacterial blight disease of barley is caused by *Xanthomonas translucens* pv. *translucens* (Jones, Johnson & Reddy) Vauterin, Hoste, Kersters & Swings (syn. *X. translucens*). The disease produces small, pale green spots or streaks on the leaves, which soon appear water-soaked. Although there is a lack of detailed information on the damage of the crop, it was suggested that since the disease often occurs on flag leaf, the yield losses may reach 10–15%. Another disease, bacterial leaf blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* is a serious disease. The disease is of epidemic potential and causes yield losses up to 20% or more in severely infected field (Mew 1993). *X. oryzae* pv. *oryzae* is reported to survive on seed, plant

debris, wild rice, and weeds, and they may serve as the primary sources of inoculums to cause bacterial leaf blight disease in rice. It is a plant quarantine pest and is subjected to phytosanitary regulations.

6.2.4 Bacterial Blight of Cotton

Bacterial blight of cotton caused by *Xanthomonas citri* pv. *malvacearum* is also seed-borne in nature and has the potential to damage the crop seriously. The disease causes yield losses in excess of 10% in the past, although significant losses due to this disease in the various countries have not been reported since many years.

6.3 Detection of Seed-Borne Bacterial Pathogens

The criteria for the selection of a technique to detect pathogen from seeds and planting materials depends upon the purpose of the test like seeds are to be tested for seed certification, seed treatment, quarantine, etc. If the seeds are tested for quarantine purposes, highly sensitive methods are required which can detect even a very less number of bacterial inoculum. Various methods are employed for the detection of seed-borne diseases such as classical methods, nucleic acid- and protein-based techniques, and spectroscopic and imaging techniques. The advanced techniques like enzyme-linked immunosorbent assay (ELISA), lateral flow device, polymerase chain reaction (PCR), and radiographic assay have been developed to detect bacterial pathogens from seed lots. A radiographic assay of seed provides an efficient, non-destructive method of seed testing, whereas ELISA, LAMP, and PCR methods are highly specific, more sensitive, and less time-consuming with higher accuracy.

6.3.1 Bioassay

Bioassay (biological assay) is a measurement of the effects of a substance on living organisms. This is an oldest seed health assay, which is widely used. It is accepted as definitive to know the infection status of a seed lot based on expression of symptom on the test plants. A positive result is usually true evidence to show the presence of viable and pathogenic bacteria. This method is highly specific to a particular host-pathogen interaction. Sensitivity of this method is variable because its inoculum's thresholds may vary depending on the plant genotypes, fluctuations in environmental conditions, fertility of soil, and many other factors (Gitaitis and Walcott 2007). The ability of plant inspectors determines detection level of the disease even at low incidences of disease. Thresholds of infection for the majority of phytobacterial diseases demand detection of 1 infested seed in 10,000 seeds. For disease development, optimum environmental conditions are required to ensure effective bioassay. In bioassay, cross-contamination from other inoculum sources can be checked by using a commercial greenhouse potting mixture or steam-sterilized soil

and creating conditions which are optimal for seed germination and seedling emergence. A minimum of appropriate sample size 30,000 seeds or 10% of seed lot should be chosen.

6.3.2 Bacterial Ooze Test

It is a common test to diagnose the disease caused by bacteria. Oozing is most prominently observed in vascular bacterial disease. This water streaming test (ooze test) is of presumptive diagnostic value in the field. A common sign of bacterial wilt disease of solanaceous crops and many more crops caused by *Ralstonia solanacearum* can be observed at the surface of freshly cut sections from severely infected stems as sticky, milky-white exudates. It indicates the presence of dense masses of bacterial cells in infected vascular bundles and especially in the xylem vessels. Bacterial ooze may also accumulate on the cut surface of the infected part particularly in potato tuber. The ooze is usually an almost pure culture of *R. solanacearum*, which can be cultured on CPA, TTC, and SMSA. This only occurs with bacterial wilt and not with any other type of pathogen or abiotic cause.

6.3.3 Bacteriophages

By using bacteriophages as detection and identification tool for seed-borne bacteria, some success has been obtained. Sutton and Katznelson (1953) reported that strain-specific and polyvirulent bacteriophages are capable to lyse target pathogenic bacteria when added to ground seeds suspended in nutrient broth. Bacteriophages were increased by increasing the plaque-forming units (PFU) over the number of PFU resulting from uninfested seeds. It indicates that a suitable bacteriophage host was present in the seed. The same approach was also followed by Yuanbo et al. (1983) to detect *X. oryzae* pv. *oryzae* in rice seeds. First rice seeds were immersed in sterile water, and then the seed extract was concentrated by centrifugation. Approximately 3–4 × 10^4 PFU/ml were added into the supernatant and again concentrated by centrifugation. The pellet was resuspended in a buffer solution and allowed bacteriophages for short period to incubate for infection of the target bacterium. Finally, the sample was concentrated and then assayed for PFU/ml.

One prerequisite to use bacteriophages in a seed health testing is that the target bacterial cells must be viable to support growth of bacteriophages. However, on the other hand, it has disadvantages as follows:

(i) Difficulty in isolating the target bacterial pathogen for confirmation.
(ii) Multiple lysotypes of the pathogenic bacterium may be present in the seed extract which may not be susceptible to the bacteriophage.
(iii) Target bacteria can exist in a lysogenic form which makes them immune against bacteriophages.

6.3.4 Semi-Selective Media

Semi-selective media are often used for the isolation and identification of particular bacteria from seeds. The media should be decided based on nutritional requirement and physiological tolerances of the target bacterium. This includes choosing a suitable carbon source that only allows growth of the target organism. Similarly, the selection of nitrogen source must support growth of the target bacterium, whereas minimize the growth of non-targets bacteria (Table 6.2, Fig. 6.1). A semi-selective medium has a higher mean plating efficiency than a common growth medium because common media have more complex nutrients and often become toxic to bacteria, perhaps due to the accumulation of peroxides.

Table 6.2 List of semi-selective medium for each specific pathogen

Primary target	Medium	References
Acidovorax avenae subsp. *avenae*	SNR	Saettler et al. (1989)
Burkholderia glumae	CCNT	Kawaradani et al. (2000)
Clavibacter; *Rhodococcus* spp.	D2	Kado and Heskett (1970)
Clavibacter michiganensis	mSCM	Waters and Bolkan (1992)
Clavibacter nebraskensis	mCNS	Shepherd et al. (1997)
Pantoea ananatis	PA20	Goszczynska et al. (2006)
Pantoea stewartii	NBY-CRN	Menelas et al. (2006)
Pseudomonas spp.	S1 & S2	Gould et al. (1985)
P. savastanoi pv. *phaseolicola*	MSP	Mohan and Schaad (1987)
P. syringae pv. *tabaci*	BCBRVB	Braun-Kiewnick and Sands (2001)
P. syringae pv. *porri*	KBC	Koike et al. (1999)
P. syringae pv. *tomato*	VBTar	Cuppels and Elmhirst (1999) and Cuppels et al. (1990)
Ralstonia solanacearum	SMSA, TTC	Elphinstone et al. (1996) and Singh et al. (2014b)
Xanthomonas spp.	Tween A, B, C	McGuire et al. (1986)
X. arboricola pv. *juglandis*	BS	Mulrean and Schroth (1981)
X. arboricola pv. *pruni*	XPSM	Civerolo et al. (1982)
X. axonopodis pv. *allii*	NCTM1	Roumagnac et al. (2000)
X. axonopodis pv. *malvacearum*	PSA+	Mehta et al. (2005)
X. axonopodis pv. *phaseoli*	Milk Tween	Goszczynska and Serfontein (1998)
X. axonopodis pv. *vignicola*	CCM	Wydra et al. (2004)
X. campestris pv. *campestris*	SM	Chun and Alvarez (1983)
X. campestris pv. *campestris*	SX	Schaad and White (1974)
X. hortorum pv. *vitians*	MMG	Toussaint et al. (2001)
X. oryzae pv. *oryzae*	mW2	Yuan (1990)

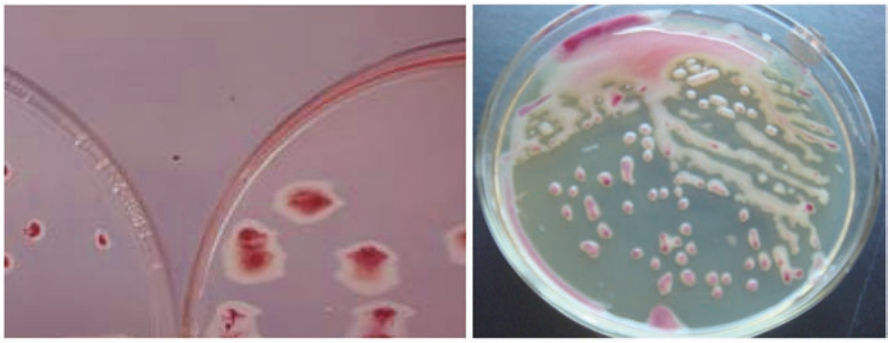

Fig. 6.1 Colony of *Ralstonia solanacearum* on SMSA (**a**) medium and TTC medium (**b**)

6.3.5 Serological Methods

Various serological techniques such as agglutination tests, immunofluorescence microscopy, immunofluorescence colony-staining, enzyme-linked immunosorbent assays, western blot, lateral flow devices, flow cytometry, and immunocapture technique (immunomagnetic separation) are used to detect and identify bacterial pathogens from different sources including seeds and plant propagating materials (Alvarez 2004).

6.3.5.1 Immunofluorescence Microscopy (IF)

This technique has been used to detect *X. campestris* pv. *campestris* in crucifer seeds (Schaad 1978) and tomato seeds for the detection of *Clavibacter michiganensis* subsp. *michiganensis*. It is difficult to establish a threshold of positive fluorescent cells which can lead to disease development in the crop. It was discouraged to use IF in the black rot seed certification program in some country like Georgia. However, it was concluded that IF showed too many false positives; it may be because of the binding of the pathogenic bacteria to non-viable cells or naked antigenic determinants.

6.3.5.2 Immunofluorescence Colony-Staining (IFC)

IFC method was developed to solve the problem of potential false positives (Franken and van Vuurde 1990). In short, an IFC seed health assay adds seed extract with an equal volume of agar medium. The mixture is incubated and dried. Thereafter, it is exposed to target pathogenic bacterium-specific antibodies conjugated with a fluorescent dye. The colonies stained with the antibody-dye conjugate can be visualized with fluorescent microscopy. The bacteria present inside the colonies can be isolated with a glass capillary tube, and then it is transferred to a suitable growth medium.

6.3.5.3 Enzyme-Linked Immunosorbent Assays (ELISA)

Now-a-days ELISA is used often for seed health assays. In general, ELISA, like the IF, also showed too many false-positive results. ELISA was successfully used to detect pathogenic bacteria *Pantoea stewartii* subsp. *stewartii* in maize seeds (Lamka et al. 1991), and it was concluded that double antibody sandwich (DAS)-ELISA

with polyclonal and monoclonal antibodies was most appropriate for seed health testing. Later, the method was used to quantify populations of *P. stewartii* subsp. *stewartii* in individual seeds by constructing a response curve relating absorbance values with numbers of bacterial CFU recovered (Gitaitis et al. 2004).

6.3.5.4 Flow Cytometry

Flow cytometry (Chitarra and Van den Bulk 2003) is another promising serological technique for use in seed health assays, which automatically sorts and analyzes bacterial cells tagged with dye-conjugated antibodies while in suspension. Several parameters can thus be determined within a few minutes by measuring the degree of light scattering and fluorescence emitted by thousands of individual cells recovered from an infected seed sample. As a stream containing tagged bacterial cells passes through a flow cell, a laser beam illuminates the cells and excites the fluorescent tags attached to the antibodies. By using different fluorescent detectors, several parameters such as cell size, granularity, and cell roughness can be measured simultaneously. If used in conjunction with fluorescent probes that target key enzymes, membrane potential, or respiratory activities, flow cytometry can also determine the viability of target cells.

6.3.5.5 Immunomagnetic Separation (IMS) PCR

In this technique, a specific antibody or antigen is used to separate sequester target cells from heterogenous mixture, and immunomagnetic separation is made of small magnetic polystyrene beads coated with antibodies (Olsvik et al. 1994). After immobilization of cells with a magnet particle concentrator, the beads are washed to remove inhibitory compounds and nontarget bacterial cells. Captured bacterial cells are boiled to release template DNA and then either it is used for PCR or captured cells can be placed onto a semi-selective medium. Walcott and Gitaitis (2000) detected *Acidovorax avenae* subsp. *citrulli* from watermelon seed. They demonstrated that IMS-PCR was able to improve 100-fold detection threshold level of *A. avenae* subsp. *citrulli* as compared to conventional PCR. Similarly, IMS was used for the detection of *P. ananatis* from onion seeds (Walcott et al. 2002). A combination of IMS-PCR may reduce false-negative reactions by enhancing the efficiency and reliability of bacterial pathogen DNA for PCR from seeds. It eliminates seed compounds which are responsible for inhibiting PCR reaction.

6.3.6 Polymerase Chain Reaction (PCR)

Kary Mullis developed polymerase chain reaction in 1983. It is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group, a specific primer is required to add in the master mixer for PCR reaction to initiate the reaction. This requirement makes it possible to delineate a specific region of template sequence for specific amplification. Billions of amplicons of the specific sequence are accumulated after completion of the PCR reaction (Table 6.3, Fig. 6.2). Several workers have reported detection of bacterial pathogens by using specific genes for development of primers for PCR

Table 6.3 Name of primers and target DNA, sample treatment in the original article, variant of PCR protocol, and reference for protocols for the different genera of plant pathogenic bacteria which causes seed-borne diseases

Species/subspecies	Primer name target DNA	Variant of PCR protocol	Sample (treatment)	Reference
Acidovorax avenae subsp. *avenae*	Aaaf3/Aaar2 (external) ITS region + Aaaf5/Aaar2 (internal) ITS region	Nested, BIO-PCRs	Seed (washes enrichment)	Song et al. (2004)
Burkholderia glumae	Forward/reverse ITS region	Real-time (SBYR® Green Master Mix)	Seed washes and plant (without extraction step) seeds	Sayler et al. (2006)
	rhs family gene (YD repeat protein)	Real-time BIO-PCR		Kim et al. (2012)
Clavibacter michiganensis subsp. *michiganensis*	CMM-5/CMM-6 *Pat*-1 gene plasmid DNA	Conventional PCR	Plant tissue and seeds (DNA extraction) bacteria (boiled)	Dreier et al. (1995)
C. michiganensis subsp. *michiganensis*	CM3/CM4 DNA fragment from a cloned pathogenic isolate	Conventional PCR	Bacteria, seeds (alkaline lysis and boiled)	Santos et al. (1997)
Pseudomonas savastanoi pv. *phaseolicola*	HM6/HM13	Conventional PCR	Bacteria, seed (DNA extraction)	Prosen et al. (1993)
	Phaseolotoxin gene cluster			
P. savastanoi pv. *phaseolicola*	P 5.1/p 3.1 (external)	Nested PCR	Seed washes (untreated)	Schaad et al. (1995)
	P 5.2/P 3.2 (internal)			
	Phaseolotoxin gene cluster			
P. savastanoi pv. *phaseolicola*	PHA19/PHA95 *amtA* gene	BIO Multiplex	Seed washes (previously plated on semi-selective medium MT)	Schaad et al. (1995) and Rico et al. (2006)
	P5.1/P3.1 + P3004L/P3004R			
	Locus *phtE*			
P. savastanoi pv. *phaseolicola*	Real-time PsF-tox/PsR-tox Probe PsF-tox-286P *tox-argK* chromosomal cluster	Real-time (TaqMan)	Bacteria, seed washes, plant (untreated)	Schaad et al. (2007)

P. savastanoi pv. *phaseolicola*	HB 14F/HB 14R (*Pseudomonas*)	Conventional PCR	Seeds alkaline lysis	Audy et al. (1996)
	Phaseolotoxin gene cluster X4c/X4e (*Xanthomonas*)			
	Plasmid DNA HB 14F+HB 14R+X4c+X4e (simultaneous detection)			
Pseudomonas syringae pvs. *syringae*, *morsprunorum*, *avii*, and *persicae*	Rep-PCR, including BOX, ERIC, REP, and IS50	Conventional PCR	Sweet and sour cherry, plum, peach, and apricot	Kaluzna et al. (2012)
Ralstonia solanacearum	PCR (16S rRNA)	Conventional PCR	Tomato plant material and soil	Umesha et al. (2012)
	Hrp B	BIO-PCR		Singh et al. (2014b)
R. solanacearum	SCAR	Conventional PCR	Tobacco leaves	Gund et al. (2011)
R. solanacearum	–	Co-operational PCR	Olive plant	Bertolini et al. (2003)
R. solanacearum	PCR from FTA cards	Real-time polymerase chain reaction (PCR)	*Geranium* plants	Tran et al. (2016)
Xanthomonas (genus)	8/27	Conventional PCR	Bacteria (boiled) or seed extract	Maes (1993)
	461/477			
	16S rRNA gene			
X. axonopodis pv. *phaseoli*	X4c/X4e (*Xanthomonas*) plasmid DNA	Conventional PCR	Seeds (alkaline treatment)	Audy et al. (1996)
Xanthomonas arboricola pv. *pruni*	–	Conventional PCR	Planting material of stone fruits	Pagani (2004) and Lopez et al. (2012)
X. campestris pv. *campestris*	HrcCF2/HrcCR2 *hrcC* gene (pathogenicity associated)	ConventionalPCR	Bacteria, plant, and seeds (DNA extraction) cauliflower seed	Zaccardelli et al. (2007)
	SCAR			

(continued)

Table 6.3 (continued)

Species/subspecies	Primer name target DNA	Variant of PCR protocol	Sample (treatment)	Reference
X. oryzae pv. *oryzae*	TXT/TXT4R*IS1113* insertion element	Conventional, BIO-PCR	Pure cultures, and plant tissue (DNA extraction) or BIO-PCR from seeds (without DNA extraction)	Sakthivel et al. (2001)
	hrp gene	–	Seed & planting material	Singh et al. (2015)
X. oryzae pv. *oryzae*, *X. oryzae* pv. *oryzicola*	XOR-F/XOR-R2ITS region TXT/TXT4R*IS1113* insertion element differentiation of pathovars *oryzae* and *oryzicola* R16–1/R23-2RITS region	Conventional, BIO-PCR	Bacteria, plant (DNA extraction or BIO-PCR from seeds without DNA extraction)	Kim and Song (1996), Adachi and Oku (2000), Sakthivel et al. (2001), Anon. (2007), and Singh et al. (2015)
X. oryzae pv. *oryzae*	–	Real-time (TaqMan probe)	–	Zhao et al. (2007)
X. oryzae pv. *oryzae*	*rhs* family gene	SYBR green real-time and conventional PCR	Bacterial cell	Cho et al. (2011)
X. oryzae pv. *oryzicola*	Putative membrane protein gene	Real-time PCR	Bacteria	Kang et al. (2012)
X. vesicatoria	RST2/RST3	Conventional PCR	Seed washes (DNA extraction) (sodium ascorbate and PVPP)	Leite et al. (1994)
	RST9/RST10			
	hrpB (hypersensitive reaction and pathogenicity gene cluster)			
X. campestris pathovars *aberrans*, *armoriaceae*, *barbarae*, *campestris*, *incanae*, *raphani*	DLH120/DLH125 *hrpF* gene (Specific for *X. campestris*) + DLH138/DLH139 ITS region from *Brassica* spp. (host internal control) DHL153/DHL154 *hrpF* gene (specific for *X. campestris*) + DHL155/DHL156 ITS region and 5.8S rRNA gene from *Brassica* spp.	Multiplex-conventional multiplex-real-time (SBYR® green master mix) (fluorescently labeled probes)	Bacteria, seed washes (DNA extraction)	Berg et al. (2005, 2006), Singh and Dhar (2011) and Singh et al. (2014a)

X. campestris pv. *hordei* *X. translucens* pathovars *arrhenatheri*, *cerealis*, *graminis*, *phlei*, *phleipratensis*, *poae*, *secalis*, *translucens*, and *undulosa*	T1/T2 ITS region	Conventional PCR	Bacteria, seeds (boiled)	Maes et al. (1996)
Xanthomonas axonopodis pv. *passiflorae*	Primers (Xapas), intergenic 16S–23S rRNA spacer region	Conventional PCR	Plant material of passion fruit	Munhoz et al. (2011)
Clavibacter xyli subsp. *xyli*	–	–	Ratoon of sugarcane	Pan et al. (1998)
Xylella fastidiosa	–	Real-time PCR	Asymptomatic plant of grape	Schaad et al. (2002)
Xanthomonas axonopodis pv. *punicae*	PCR (*gyr* B)	Conventional PCR	Pomegranate leaf, fruit, stem	Mondal et al. (2012)
Xanthomonas axonopodis pv. *malvacearum*	–	Conventional PCR	Cotton	Chakrabarty et al. (2005)
E. amylovora	PCR (short sequence DNA repeat)	Conventional PCR	Raspberry fruit	Jock et al. (2003)
Dickeya sp. (*P. chrysanthemi*)	PCR (16S-23S rDNA)	Conventional PCR	Banana tissues, soil, and water	Li et al. (2011)
P. stewartii	PCR (pstS-glmS region) and qPCR	Conventional PCR	Bacteria	Wensing et al. (2010)
Erwinia amylovora, *E. pyrifoliae*	PCR (pstS-glmS region)	qPCR conventional	Apple and pear fruit	Wensing et al. (2012)
Candidatus Liberibacter asiaticus	–	Nested PCR and TaqManReg	Citrus	Lin et al. (2010)

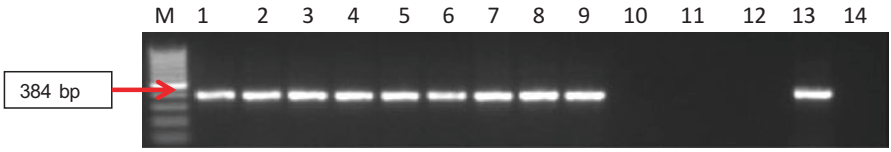

Fig. 6.2 Amplification of DNA bands of nine strains of *X. oryzae* pv. *oryzae* isolated from different locations of India after PCR amplification by using *hrp* gene-based primer at 384 bp. Lane M, 1 kb DNA ladder; lanes 1–9 and 13, 9 strains, viz., PNA, PNB, BB49, BB50, BB7, BB1, Bb2, BB40, and IARI of *X. oryzae* pv. *oryzae*; 10, *X. campestris* pv. *campestris*; 11, *X. axonopodis* pv. *punicae*; 12, *R. solanacearum*; 14, −ve

amplification (Prosen et al. 1993; Dreier et al. 1995; Santos et al. 1997; Louws et al. 1999; Schaad et al. 2003; Berg et al. 2005) or random amplified polymorphic DNA (RAPD) profiles (Meng et al. 2004) from pathogenic bacteria infested seeds. Initially PCR was considered too sensitive which can be used routinely as a seed health assay. Moreover, theoretically, it is capable to detect a single bacterial cell. The sample size and volume of seeds (e.g., 30,000 seeds/liter of buffer) are being tested in conjunction with the small volume (~4 µl) which can be used as a template in the PCR reaction and make PCR no more sensitive than many other techniques. However, there is a concern about the ability of PCR to differentiate between viable and dead cells.

6.3.6.1 Nested PCR

Nested PCR was used to detect bacterial pathogens from seeds (Ojeda and Verdier 2000). It increases the sensitivity by utilizing a second round of amplification using primers designed to anneal to internal regions of the amplicon produced by the first round of amplification. Pradhanang et al. (2000) reported potentiality of nested PCR in the field of plant pathology. Poussier et al. (2002) used nested PCR to detect *Ralstonia solanacearum* from tomato seeds. There are some problems by changing the sequences of genetic loci targeted by PCR primers. Some bean seeds escaped detection and were mistakenly certified as disease-free, because the seed lots contained *P. savastanoi* pv. *phaseolicola* strains and lacked the tox gene cluster, the site that PCR normally targets (Rico et al. 2003).

6.3.6.2 BIO-PCR

In BIO-PCR, the viable enrichment of growth media with an enzymatic amplification is combined for improvement of sensitivity of PCR amplification. First, the target bacterium is enriched in liquid or solid media, which is detected at extremely low levels in seeds and propagative materials. The following simple steps are included in BIO-PCR method such as extract a sample; plate a sample onto agar media or liquid medium; incubate for 15–72 h, depending on the growth of target bacterium; wash plates to remove bacteria or centrifuge liquid medium; and then use 1 or 10 µl for direct PCR amplification. It has several advantages as compared to conventional PCR like increased sensitivity of the primer, eliminates PCR

inhibitors, and detects viable cells only. This technique may be used in conventional as well as real-time PCRs (Lin et al. 2009; Singh et al. 2014a, b, 2015).

Schaad et al. (1995) developed BIO-PCR to ensure the detection of viable cells only. Seeds having less population of pathogenic bacterium are crushed in sterilized distilled water and then spread extracts onto the surface of a suitable growth medium to increase population of target bacterium. Then enriched plate is incubated for 24–48 h and washed. DNA extracted from this suspension is used for PCR. The living cells of target bacterium will grow only on the growth medium, which make guarantee that the bacterium was viable in the seed. However, in most of the cases, the seed lots may harbor fast-growing, saprophytic bacteria; therefore, the seed extract must be enriched on a semi-selective medium (Song et al. 2004). Several workers used BIO-PCR technique successfully to detect bacterial pathogens in the seed of different crops (Schaad et al. 1995, 2003; Sakthivel et al. 2001; Song et al. 2004; Singh et al. 2015) (Fig. 6.3).

Bacterial blight pathogen *Xanthomonas axonopodis* pv. *punicae* was detected from leaf, fruit, etc. parts of pomegranate through PCR using primers designed from *gyrB* gene amplified at 530 bp. A primer set KKM5 and KKM6 was synthesized based on sequence alignment of 530 nucleotides of C-terminus region in the *gyrB* genes from 15 different bacterial strains. The primer set was validated for the amplification of 491 bp of *gyrB* gene. No amplification was observed in other phytopathogenic *Xanthomonas* and *Pantoea agglomerans* (Mondal et al. 2012).

6.3.6.3 Real-Time (Quantitative) PCR

Real-time PCR is now becoming very useful because of the development of detectors which can measure fluorescence emitted during the PCR cycle. The technique depends on the 5′– > 3′ exonuclease activity of Taq DNA polymerase, resulting in cleavage of fluorescent dye labeled with different probes (TaqMan®) during PCR. In theory, the exponential nature of PCR allows the amount of starting material to be calculated from the amount of product at any point in the PCR reaction. However, PCR reaction conditions can intervene with exponential amplification as well as affect concentration of end product. Several plant pathogenic bacteria have been detected in plants including *Clavibacter michiganensis* subsp. *sepedonicus* and *R. solanacearum* from potato tubers, *Agrobacterium* strains from plants, and

Fig. 6.3 Amplification of *hrp* gene DNA fragment at 384 bp of *X. oryzae* pv. *oryzae* detected from artificially inoculated rice plants subsequently stored seeds of rice up to 8 months under ambient conditions by using Bio-PCR. Lane M, 100 bp of DNA ladder; lane 2, +ve control (DNA of *X. oryzae* pv. *oryzae*); lanes 2–11, DNA template used as bacteria extracted from seeds allowed to grow in the nutrient broth and then DNA extracted using shortcut method; lanes 12–21, DNA template used for PCR as bacteria extracted from seeds and allowed to grow in nutrient broth; lanes 22–31, DNA template used as suspension of bacteria in brine solution extracted from seeds

Xylella fastidiosa from asymptomatic grapevines by using real-time PCR. Real-time PCR enables the detection and quantification of pathogens and carries off the labor and hazardous waste issue related to gel electrophoresis.

SYBR Green I has become the most popular and widely used DNA dye for the detection of bacteria by real-time PCR due to its cost efficiency, generic detection of amplified DNA, and ability to distinguish PCR products by analysis of melting curve. However, SYBR Green I has some disadvantages also like it inhibits PCR amplification in a concentration-dependent manner, effects on DNA melting temperature and preferential binding to certain DNA sequences.

Burkholderia glumae was detected in rice seeds by using real-time PCR technique (Sayler et al. 2006). Maeda et al. (2006) used multiplex PCR when more than one plant bacterial target was associated with a seed lot. Multiplex PCR technique may be found more effective to use real-time PCR via the use of multiple TaqMan® probes with fluorophores that emit light in different wavelength ranges. To detect and quantify pathogen populations by using quantitative real-time PCR holds great potential for studying the epidemiological significance of seed-borne inoculums. It is also able to establish and monitor seed transmission thresholds for improvement of disease management.

6.3.6.4 Rapid-Cycle Real-Time PCR

PCR-based seed detection assays adopted by commercial and government seed testing agencies are slow due to the high cost of the equipment and consumables and lack of technical experts to conduct the assay. Moreover, cross-contamination risks as well as gel electrophoresis step have made the methods untempting. Rapid-cycle real-time PCR is an advanced technique in the form of promise to remove many barriers as reported earlier in conventional PCR and make it more accessible to detect bacterial pathogen from seed and propagating materials. In real-time PCR, DNA amplification is coupled with the production of a fluorescent signal that increases proportionally with the numbers of amplicons produced (Kurian et al. 1999; Cockerill and Smith 2002). Reporter and quencher dye molecules are synthesized in TaqMan probes at the 5′ and 3′ ends, respectively. There is no fluorescence in this configuration, but when reporter and quencher dye are detached, the reporter dye fluoresces. In TaqMan system, the annealing of a complementary probe to the template DNA is a first step in the PCR. Taq DNA polymerase has 5′ exonuclease activity (cleaves off nucleotides from the 5′ end of nontemplate complement DNA). The TaqMan probe is excised during the extension step of PCR, detaching the reporter dye from the quencher molecule. This results in fluorescence that is detected by photosensors. The intensity of fluorescence is directly related to the excision of reporter dye molecules that is directly related to amplification of DNA. Cockerill and Smith (2002) reported fluorescent resonance energy transfer (FRET) and molecular beacon probes for the detection of bacteria which are also employed for real-time PCR. Real-time PCR has several key advantages over conventional PCR as:

1. Reduces DNA amplification time due to rapid cycling.
2. Lessens the risk of cross-contamination.

3. To see the PCR result, post-PCR electrophoresis is not required.
4. Probes can allow for multiplex PCR, to detect multiple pathogens in the same reaction by using different dyes (Wittwer et al. 2001).
5. Quantifies template DNA through real-time PCR to determine levels of seed infestation.

However, there are some disadvantages like higher cost of equipment and consumables and inhibition by seed-derived compounds to prevent the immediate adoption of this technology for seed detection. Therefore, still it is required to implement strategies upstream of PCR, which produce template DNA/RNA of PCR quality. Sensitivity of detection level can significantly be improved by combining real-time PCR with the other methods like MCH-PCR, IMS-PCR, and BIO-PCR. Zhao et al. (2009) detected *A. avenae* subsp. *citrulli* in watermelon seeds by using real-time PCR.

6.3.6.5 Multiplex PCR

In multiplex PCR, different DNA targets of more than one pathogen are detected in a single reaction from a sample simultaneously. This technique is very useful in plant pathology because different groups of pathogens like bacteria, viruses, fungi, and phytoplasma frequently infect a single host. Hence, to save time and money, multiplex PCR is required to produce disease-free seed and other propagating plant materials. There are several examples of simultaneous detection of several targets of the pathogens which used multiplex PCR.

A multiplex polymerase chain reaction (PCR) protocol was developed for simultaneous detection of *R. solanacearum* causing brown rot (wilt) and *E. carotovora* subsp. *carotovora* incited soft rot diseases in potato tubers (Ranjan et al. 2016). They designed the primers' set targeting the pectate lyase (pel) gene of *E. carotovora* subsp. *carotovora* and the universal primers based on 16S r RNA gene of *R. solanacearum* (Fig. 6.4) and standardized multiplex PCR protocol to detect *R. solanacearum* and *E. carotovora* subsp. *carotovora* up to genomic DNA 0.01 and 1.0 ng, respectively. The method offers sensitive, specific, reliable, and fast detection of two major bacterial pathogens from potato tubers simultaneously.

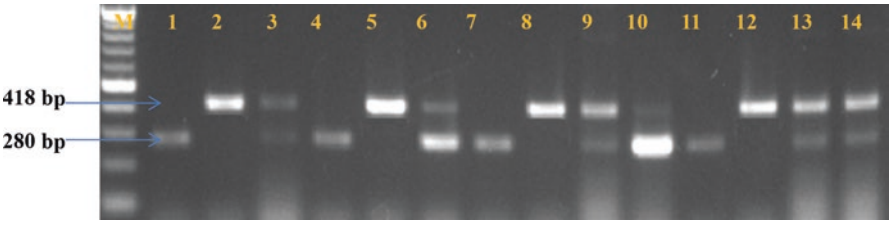

Fig. 6.4 Validation of multiplex PCR for the detection of *R. solanacearum*, *E. carotovora* subsp. *carotovora*, and both *R. solanacearum* and *E. carotovora* subsp. *carotovora* from potato tubers collected from different agro-climatic regions. Lane M, 100 bp DNA ladder; lanes 1–3 Uttarakhand; 4–6 Odisha; 7–10 Delhi; and 11–14 Meghalaya. Source: Ranjan et al. (2016)

Bacterial blight of onion caused by *Xanthomonas axonopodis* pv. *allii* is an emerging disease and transmitted through seed. Isabelle et al. (2010) designed primers using two RAPD and AFLP sequences corresponding to pilus assembly genes like *pilW* and *pilX* and the *avrRxv* gene, respectively, to develop protocol of multiplex nested PCR. This technique was used for the detection of *X. axonopodis* pv. *allii* strains pathogenic to onion and/or other *Allium* species isolated in different regions of the world.

A multiplex PCR protocol has been developed for simultaneous, specific, and rapid detection of *R. solanacearum* and *X. perforans* from plant materials (Umesha and Avinash 2015). They used species-specific primers RS-F-759 and RS-R-760 for *R. solanacearum* and RST2 and RST3 for *X. perforans* amplified at 281 and 840 bp, respectively. Choi et al. (2010) have developed a biomarker system which simultaneously checks *Xylella*-induced plant transcripts and ribosomal (r)RNA of *Xylella*. Plant biomarker genes were obtained from a combination of in silico analysis of grape expressed sequence tags and validation by means of RT-PCR. In this case, four genes were upregulated upon Pierce's disease infection, and they were individually multiplexed with a *Xylella fastidiosa* marker rRNA. The protocol was enough sensitive to detect both pathogen rRNA and host gene transcript in asymptomatic infected grape plants.

6.3.6.6 Restriction Fragment Length Polymorphisms (RFLP)

It is a slightly old method to use for the detection and differentiation of strains/pathovars of bacterial pathogens, and RFLP product is observed after digestion with *Pst*1 which showed specificity for a group of a strain within a pathovar. Leach and White (1991) distinguished strains of *X. oryzae* pv. *oryzae* causing bacterial leaf spot of rice occurring in the Philippines and United States. They reported that the strains of the United States are not closely related to the Asian strains due to their differences in RFLP patterns. Yashitola et al. (1997) reported that *X. oryzae* pv. *oryzae* occurred in 16 of 18 locations in India which belonged to the pathotype 1b by using the RFLP analysis.

6.3.6.7 Fluorescent In Situ Hybridization (FISH)

In this technique 20–30 mers nucleotide probes are applied against 16S or 23S rRNA/DNA. These nucleotide probes are used for in situ hybridization, because they are able to diffuse through the cell wall of bacteria which are present in thin tissue sections or in plant or soil extracts fixed on a microscopic slide. FISH technique using probes targeted to 23S rRNA are applied to detect *R. solanacearum* race 3 bv 2 from potato peels. Wullings et al. (1998) developed a probe specific for *R. solanacearum*, and it was used to identify the pathogen in potato samples accurately and further tested simultaneously and independently by using pathogen-specific immunofluorescence method.

6.3.6.8 Magnetic Capture Hybridization and PCR (MCH-PCR)

MCH-PCR is a relatively new technique which was first described in 1995 for the detection of *Pseudomonas fluorescens* in nonsterile soil (Jacobsen 1995). This

technique is similar in format to IMS-PCR; however, MCH-PCR uses single-stranded DNA probes to capture and concentrate specific DNA fragments. Then it can be used as templates for PCR. This technique has subsequently been developed for the detection of fungi, viruses, and bacteria in water, wood, soil, and food, all of which contain PCR-inhibitory compounds (Chen et al. 1998; de Moraes et al. 1999; Chen and Griffiths 2001; Langrell and Barbara 2001). Despite its great potential for the detection of bacteria, however, MCH-PCR has not been used so far for the detection of seed-borne bacterial pathogens (Nielsen et al. 2002).

6.3.7 Loop-Mediated Isothermal Amplification (LAMP)

This technique has been proved to be the best approach for amplifying nucleic acid with high accuracy, specificity, sensitivity, and rapidity. In this technique, thermocycler is not needed for detection of the bacteria. The principle is based on strand displacement activity followed by amplification using a unique DNA polymerase. A set of four specially designed primers targeting the *R. solanacearum* fliC gene coding for flagellar proteins was designed which recognized a total of six distinct sequences on the target DNA. Amplification performed for 60 min at 65 °C resulted in the production of magnesium pyrophosphate, which increased the turbidity of the solution, permitting visual assessment (Kubota et al. 2008). The LAMP product was detected only in samples containing *R. solanacearum* in mixed cultures of different bacterial pathogens. *Rhodococcus fascians* causes leafy galls and other plant distortions resulting in economically significant losses to nurseries producing in ornamental plants. Serdani et al. (2013) detected pathogenic *R. fascians* from naturally infected plants by using LAMP technique. The detection limit of LAMP was 10^3 CFU/30 µl reactions. The LAMP assay is a significant advancement in rapid diagnostics of *R. fascians.* The technique is also useful where limited laboratory facilities are available to confirm the presence of this pathogen in infected plants.

6.4 Conclusion

In bioassay, highly favorable environmental conditions are required during seedling establishment process for the disease development. Hence, it is critical to ascertain that potentially damaging pathogens are not introduced on seeds. It may be most effectively completed by eradication, using seed detection method to identify the contaminated seed lots which can then be disposed or treated. Traditional seed detection techniques such as visual observation, selective media, serological method, and bioassay are used extensively. But these methods have shortcomings ranging from inefficiency to lack of specificity and sensitivity. PCR has great potential to improve bacterial pathogen detection in seeds, as it substantiates many of the important characteristics such as specificity, sensitivity, rapidity, ease of implementation, interpretation, and applicability, further increasing sensitivity and reducing inhibitory seed compounds by using BIO-PCR, IMS-PCR, and MCH-PCR are

beneficial possibilities. IMS-PCR and MCH-PCR are particularly attractive because they provide simple and universally applicable formats for testing seeds for different culturable bacterial pathogens. Like other fields in which pathogen detection is critical, seed detection method must be based on new technologies. However, before adopting new technologies, it is important to rigorously evaluate their applicability, sensitivity, precision, and accuracy in real-world. Before going for commercialization of new techniques, they must be validated in stringent multi-laboratory tests that examine their reproducibility and repeatability. Little is known about their applicability for routine seed testing.

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Detection and Diagnosis of Seed-Borne Viruses and Virus-Like Pathogens

7

Sumit Inder Kaur, Prem Lal Kashyap, Santokh Singh Kang, and Abhishek Sharma

Abstract

Quick and reliable detection and diagnosis of virus infection in agricultural crops are critical to prevent the spread of the virus and check yield losses, because at several occasions, infected seed materials seem symptomless. Seed diagnosis can shun hysterical and long-distance movement of viruses through seed and planting material. Additionally, this will control injudicious application of agrochemicals and the introduction of health hazardous toxic chemicals into the environment. Several approaches based on the biological, biochemical, immunological, and nucleic acid properties of viruses have been developed for the reliable, sensitive, and specific detection and differentiation of viruses and their strains in seeds. The most widely used method is conventional PCR, and presently, improved versions of PCR-based assays such as nested PCR, multiplex PCR, and real-time PCR have been reported to detect seed-transmitted viral load in various crops. Recently, loop-mediated isothermal amplification (LAMP) and next-generation sequencing (NGS) have been explored for their application in diagnostics of seed-borne viruses. Briefly, the present chapter outlines and discusses the conventional and innovative diagnostic techniques that have been reported to monitor and identify the seed-borne viruses in different food crops.

Keywords

Detection · Diagnosis · PCR · Seed · Virus · Vegetables

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7.1 Introduction

A virus is one of the most imperative disease-inciting agents in different kinds of seeds and planting materials used in agriculture. Seed-borne viruses (SBV) are reported to be the most efficient means of dissemination for a number of viruses and cause devastating yield and financial losses (Table 7.1). Principal seed abnormalities which occurred due to virus infection include rapid decline in yield and seed germination, alterations in seed shape and color, etc. (Mandhare and Gawade 2010; Inoue-Nagata et al. 2016). Fletcher et al. (1969) documented 15% yield loss in cucurbits, when *Cucumber green mottle mosaic virus* (CGMMV) attacked at an early growth phase. Similarly, *Pea seed-borne mosaic virus* (PSbMV) also has the potential to cause substantial yield losses when all plants are infected early (Ali et al. 1998). Enhanced yield losses would also occur due to mixed invasion of two or more viruses in synergistic mode rather than individual virus infection (Sastry 2013). SBV also showed indirect effects by influencing the cost of chemicals employed for the management of virus vectors and production of virus-free planting and seed materials (Hull 2009; Sastry and Zitter 2014). Moreover, the lack of effective chemical management module for plant viruses along with farmers' inadequate understanding regarding the viruses makes it difficult to avert monetary losses (Erkan 1998). In addition, the abrupt appearance and incidence of viral diseases in agricultural fields depend largely on the positive correlation of virus, host, vector, and environment (Allam et al. 1980).

Seed transmission is one of the major ways for the dissemination and survival of a myriad of agriculturally important plant viruses (Johansen et al. 1994). Infected seed is the most important source of viruses in commercial production. There are three principal routes of vertical movement of viruses via seed contamination (Fabre

Table 7.1 Economic yield losses due to major seed-transmitted viruses (STV) in different crops

S. no.	Virus	Crop	Yield loss (%)	Reference
1	*Barley stripe mosaic virus*	Barley	35–40	Eslick (1953)
2	*Lettuce mosaic virus*	Lettuce	100	Broadbent et al. (1951), Grogan et al. (1952), and Zink et al. (1956)
3	*Cowpea mosaic virus*	Cowpea	13–87	Kaiser and Mossahebi (1975)
4	*Cowpea mosaic virus*	Cowpea	64–75	Suarez and Gonzalez (1983)
5	*Pea seed-borne mosaic virus*	Pea	16–78	Kraft and Hampton (1980)
6	*Pea seed-borne mosaic virus*	Pea	18–25	Coutts et al. (2009)
7	*Potato virus Y*	Potato	10–100	Warren et al. (2005)
8	*Pepper mild mottle virus* (PMMoV)	Pepper	100	Green (2003)
9	*Cucumber mosaic virus* (CMV)	Cowpea	14	Pio-Ribeiro et al. (1978)
10	*Potyvirus* and *Crinivirus*	Sweet potato	80–90	Mukasa et al. (2006)

et al. 2014). For instance, stable viruses like *Tobamovirus* reside only in the seed coat without embryo contamination and later transmitted to the seedling after germination (Broadbent 1965). Other methods of seed contamination that correspond to virus invasion of the embryo include infected maternal tissues and pollen (Fabre et al. 2014). Approximately, 231 plant virus and viroid diseases have been established as seed-transmissible (Kil et al. 2016). From tomato crop, *Tomato black ring virus* (TBRV), *Pepino mosaic virus* (PepMV), *Tomato chlorotic dwarf viroid* (TCDVd), *Arabis mosaic virus* (ArMV), *Tomato mosaic virus* (TMV), *Tomato apical stunt viroid* (TASVd), and *Tomato streak virus* (TSV) were identified as STV (Córdoba-Sellés et al. 2007; Kil et al. 2016). Among them, TCDVd showed 85.5–94.4% transmission through seeds in tomato, and parallel levels of seed transmission were also reported from TASVd (80%) and TSV (66%) viruses (Berkeley and Madden 1932; Antignus et al. 2007). Further, *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic virus* (SqMV), *Tobacco ring spot virus* (TRSV), and CGMMV are also reported as SBV throughout the globe (Provvidenti 1996; Sevik and Balkaya 2015). Seed coat infection of CMV differs from 53 to 83%; however, it falls between 10 and 46% in the case of seed embryo (Ali and Kobayashi 2010). Several reports indicated that virus-infected seeds can be noticed in every corner of the globe even in the crop raised from certified seed and planting material (Albrechtsen 2006; Gumus and Paylan 2013). In the case of viroids, 0–100% seed transmission rates have been reported for *Potato spindle tuber viroid* (PSTVd) in tomato and potato (Simmons et al. 2015).

Seed-borne viruses (SBV) in different crops can be managed by an integrated approach that encompasses healthy planting/seed material, host resistance, isolation, sanitation, elimination of plant reservoirs of viruses (e.g., weeds or volunteer plants), crop rotation, host-free periods, cross-protection, virus and vector avoidance by altering date of sowing and harvesting, control of insect-virus vectors through pesticides, trap or border crops, sticky traps, netting, reflective mulches, and rouging (Jones 2006). Control of virus spread is obligatory, because there is no other alternative to cure greenhouse- or field-grown plants after viral infection. Therefore, there is an imperative necessity to devise sensitive, trustworthy, and rapid assays for the detection of STV in plants, usually which may be present at a low concentration, and also the host crop may be a symptomless carrier. Currently, there is no worldwide acceptable protocol for the detection of SBV, and the principal assays developed so far for their detection and diagnosis can be categorized as physical-, biological-, serological-, and nucleic acid (molecular)-based methods (Table 7.2). Bioassays which relied on the symptoms produced by viruses in experimentally infectable plant hosts offer preliminary information for the detection and diagnosis of viruses and viroids (Albrechtsen 2006). However, these biological methods are laborious and entail a lot of space. Physical methods like electron microscopy (EM) of planting and seed material are more briskly and provide tentative identification which can be later validated by employing other criteria. Unfortunately, these assays require expensive instrumentation which is usually hard to upkeep in good working conditions. The serological methods surmount the limitations of biological and physical methods since they exercise only minute fraction of the information encoded by the virus. In serology, a myriad of tests employing

Table 7.2 Principal diagnostic assays reported for the detection of SBV

S. no.	Technique(s)	Virus	Host	Reference
A. Biological methods				
	Grow-out, indicator plant assay	*Cowpea aphid-borne mosaic virus* (CABMV), *Cucumber mosaic virus* (CMV), and *Southern bean mosaic virus* (SBMV)	Cowpea	Salem et al. (2010)
B. Serological methods				
	Indirect ELISA	*Bean common mosaic virus*	Common bean	Mandour et al. (2013)
	DAS-ELISA	*Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic virus* (SqMV), *Tobacco ring spot virus* (TRSV), and *Cucumber green mottle mosaic virus* (CGMMV)	Winter squash	Sevik and Balkaya (2015)
	DAS-ELISA	*Pepper mild mottle virus* (PMMoV), *Cucumber mosaic virus* (CMV), *Potato virus* Y (PVY), and *Alfalfa mosaic virus* (AMV)	Pepper	Miloševic et al. (2015)
	DAS-ELISA and RT-PCR	*Alfalfa mosaic alfamovirus* (AMV), *Cucumber mosaic cucumovirus* (CMV), *Lettuce mosaic potyvirus* (LMV), *Cucumber green mottle mosaic virus* (CGMMV), *Tomato bushy stunt tombusvirus* (TBSV), *Tobacco mosaic tobamovirus* (TMV), *Tomato black ring nepovirus* (TBRV), and *Tomato mosaic tobamovirus* (ToMV)	Pepper, tomato, cucumber, lettuce	Gumus and Paylan (2013)
	TPIA and DBIA	*Potato leaf roll virus* (PLRV), *Potato virus S* (PVS), *Potato virus X* (PVX), and *Potato virus Y* (PVY)	Potato	Samsatly et al. (2014)
C. PCR-based methods				
	RT-PCR	*Soybean vein necrosis virus*	Soybean	Groves et al. (2016)
	PCR	*Tomato yellow leaf curl virus*	Tomato	Kil et al. (2016)
	PAS ELISA	*Cowpea aphid-borne mosaic virus*	Cowpea	Ojuederie et al. (2009)
	RT-PCR	*Cowpea aphid-borne mosaic virus* (CABMV), *Cucumber mosaic virus* (CMV), and *Southern bean mosaic virus* (SBMV)	Cowpea	Salem et al. (2010)
	Real-time PCR	*Squash mosaic virus* (SqMV)	Cucurbits	Ling et al. (2011)
	Macroarray	*Alfalfa mosaic virus*, *Cucumber mosaic virus*, *Potato mop-top virus*, *Potato leafroll virus*, *Potato latent virus*, *Potato virus A*, *Potato virus M*, *Potato virus S*, *Potato virus X*, *Potato virus Y*, and *Tobacco rattle virus*	Potato	Agindotan and Perry (2008)

(continued)

Table 7.2 (continued)

S. no.	Technique(s)	Virus	Host	Reference
	Duplex real-time PCR	*Squash mosaic virus* (SqMV) variants	Squash	Li et al. (2016)
	cDNA macroarray	*Alfalfa mosaic virus*, *Cucumber mosaic virus*, *Potato aucuba mosaic virus*, *Potato leafroll virus*, *Potato mop-top virus*, *Potato virus A*, *Potato virus M*, *Potato virus S*, *Potato virus X*, *Potato virus Y*, *Tomato ringspot virus*, and *Tomato spotted wilt virus*	Potato	Maoka et al. (2010)
	Real-time PCR	*Potato leafroll virus* (PLRV), *Potato virus X* (PVX), and *Potato virus S* (PVS)	Potato	Mortimer-Jones et al. (2009)
	Real-time PCR	PVY, PLRV, PVX, and *Potato virus A*	Potato	Agindotan et al. (2007).

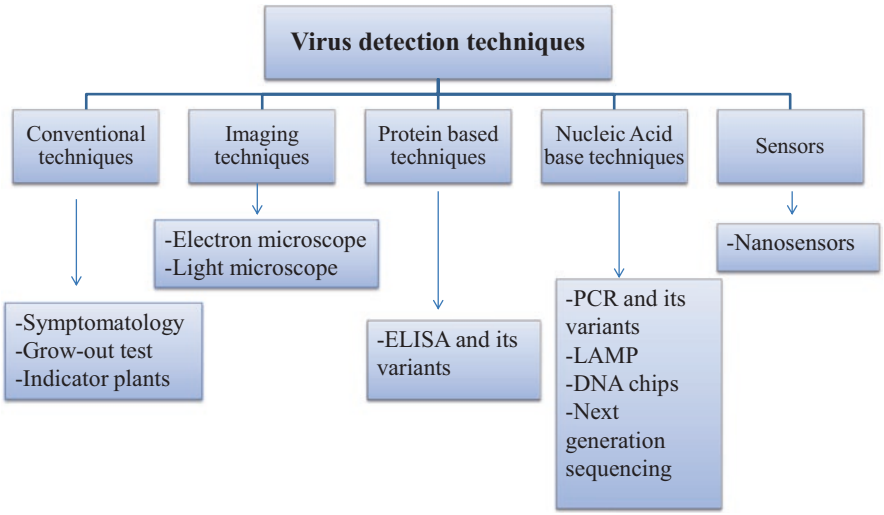

Fig. 7.1 Different techniques used for the detection of SBV

antigen-antibody recognition are available to confirm the presence of virus even at lower concentration, and in few instances, it also assisted in the detection of localized infections. Enzyme-linked immunosorbent assay (ELISA) using fluorogenic, chemiluminescent, and chromogenic substrates is one of routinely used serological assays (Samsatly et al. 2014). Molecular methodologies involving nucleic acid hybridization, which employ whole or some fraction of the viral genome, have been reported (Kashyap et al. 2016; Selvarajan and Balasubramanian 2016; Sharma et al. 2017; Kashyap et al. 2019). The aim of the present chapter is to outline the current status of various cutting-edge diagnostic techniques (Fig. 7.1) that can be applied for the diagnostics of SBV in different crops.

7.2 Conventional Virus Detection Assays

7.2.1 Symptomatology

Symptoms produced by SBV in various crops (Fig. 7.2) are commonly employed to identify and characterize a disease having viral etiology and for roguing of diseased seedlings in an effort to manage the disease. Visual examination and inspection is a fairly simple and effortless method, when characteristic symptoms of a particular viral infection are easily visible on infected plants. However, several parameters like host plant, virus strain, time of infection, and the environment can persuade the typical symptom expression (Walcott 2003). The visual examination of the seed morphology like shape, seed coats, color, size, and weight indicates the presence of virus. The virus-infected seeds are usually shrunken in shape, having shriveled and discolored seed coat, smaller in size, and light weighed (Salem et al. 2010). Sometimes, the infected seeds are cracked and may have necrotic spots on their surface. Phatak and Summanwar (1967) observed that the majority of the shriveled seeds of barley and cowpea suffered from *Barley false stripe virus* and *Cowpea mosaic virus* (CPMV) infection, respectively. Moreover, later exhaustive

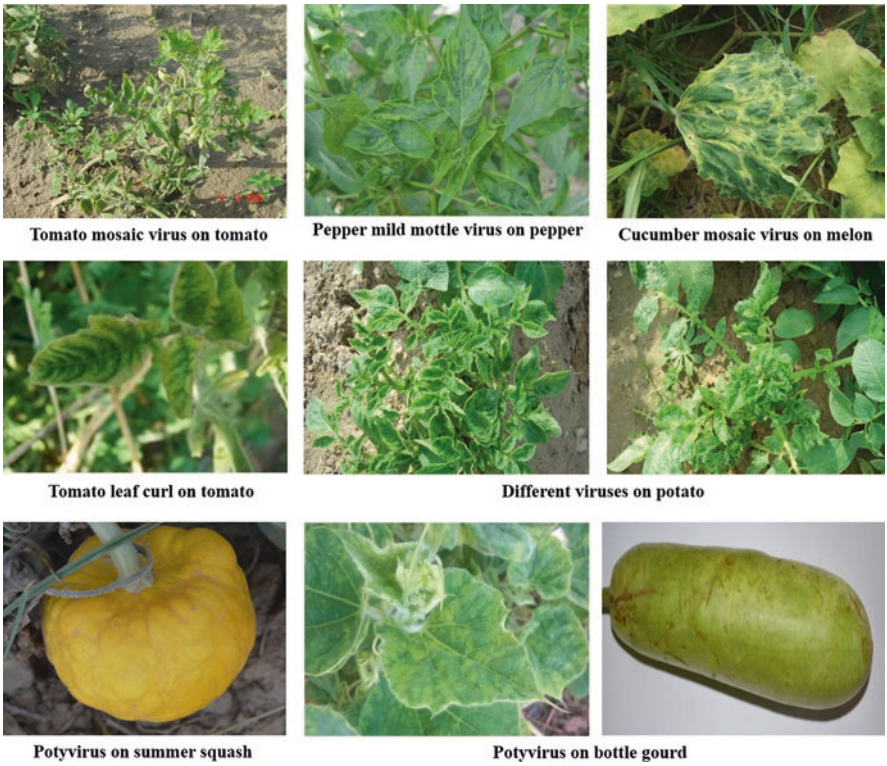

Fig. 7.2 Symptoms produced by seed-borne viruses in various crops

experimentation on the morphology of seed coat and virus transmission clearly revealed that the seed coat mottling by *Bean pod mottle virus* (BPMV) and *Soybean mosaic virus* (SMV) resulted into infected soybean plants when grown (Hobbs et al. 2003). Phatak and Summanwar (1967) reported significant seed transmission of CPMV in the shriveled seeds of cowpea. Coutts et al. (2009) observed necrotic rings and line markings on seed coat malformation in the seeds of *Vicia faba* infected with PSbMV. Besides this, there are few examples wherein mottled and non-mottled seeds of soybean due to SMV infection have been noticed (Chalam et al. 2004; Parakh et al. 2005). The major drawback of this detection method is that sometimes the visual morphological observation may provide wrong impression of virus infection. For instance, Andayani and associates (2011) documented no significant association or linkage between SMV transmission and mottling of soybean seeds. Similar observations have been made by Pacumbaba (1995) and Bazwa and Pacumbaba (1996). Moreover, it is clearly evident that viral infection is not associated with the mottling of soybean seeds, which may be due to environmental factor or any hereditary trait (Porto and Hagedorn 1975). Similarly, Stevenson and Hagedorn (1970) also pointed out that seed coat cracking in pea seeds was not related with *Pea seed-borne mosaic virus* (PSbMV) infection. Later, ELISA-based experimentation with SMV in soybean (Chalam et al. 2004) showed that the several times mottled soybean seeds were free from infection, in contrast to healthy-looking seeds showed SMV infection. In addition, dissimilar viruses can show analogous symptoms or diverse strains of a virus can cause distinct symptoms in the same host. While symptoms offer fundamental information on viral diseases, sufficient field information is needed when drawing any conclusion on symptomatology alone. Usually, it is crucial that visual examination for symptoms in the agricultural fields is conducted in juxtaposition with other confirmatory assays to ensure precise and correct identification of SBV. Additionally, the accurateness and consistency of these methods relied heavily on the acquaintance, experience, and skill of the individual making the diagnosis (Alvarez 2004; Kashyap et al. 2011).

7.2.2 Grow-out Test

Grow-out test is done to check the percent seed transmission of viruses in the infected seeds based on the visual characteristic symptoms produced by the particular host-virus interaction. For this purpose, infected seeds are sown in sterile soil under controlled glasshouse environment either in Petri plates or trays or in moist towel papers. The newly emerged leaves of the seedlings are monitored for characteristic and prominent symptoms that appeared according to a particular virus-plant association. Popularly, germinating and growing seed material in glasshouse for the purpose of virus identification and detection is also termed as sand bench germination test. Rohloff (1967) detected the presence of LMV in lettuce by grow-out test after 2–3 weeks when seedlings displayed typical mosaic symptoms. Later, similar experimentation to confirm the presence of PSbMV has been executed by growing

seeds under glasshouse conditions (Naim and Hampton 1979; Hampton et al. 1981). Tosic and Pesic (1975) reported that *Alfalfa mosaic virus* (AMV) can be detected in 1-week-old alfalfa seedlings at cotyledon stage by performing grow-out tests. Phatak 1974 recorded characteristic and peculiar symptoms of BSMV in 7-day-old barley seedlings. He also demonstrated a "blotter test" in Petri dishes for the detection of nepovirus infection in *Petunia violacea.* The results of the developed assay were later validated by grow-out test as well as by sap inoculation for the confirmation of TRSV infection (Phatak (1974). Puttaraju and associates (1999) have demonstrated an established grow-out test for the detection of *Bean common mosaic virus* (BCMV) in French bean seed collections. Similarly, by conducting grow-out assays, the presence of different STV in cowpea and French bean seeds has been reported by Pena and Trujillo (2006) and Udayashankar et al. (2010). Seedling grow-out is one of the most applicable and widely used seed detection assays, but for successful implementation, infected seedlings must display obvious and characteristic symptoms (Sastry 2013). The seedling grow-out assay is also laborious, requiring up to 3 weeks for seedling germination and symptom development. Finally, seed test personnel must be familiar with the symptoms associated with each seed-borne viral infection. This can be difficult since each viral infection has a range of possible symptoms that are influenced by environmental conditions. Hence, for the seedling grow-out assay, greenhouse conditions must be strictly regulated to ensure consistent results.

Embryo culture technique (ECT) is another promising technique for the rapid identification and detection of viruses in planting material and seed lots of crops during the off season (Sastry 2013). This method involves artificial culturing of embryo after its excision. The artificial culturing of rescued embryo also permits the virus to grow rapidly up to a detectable concentration. For the first time, Crowley (1957) used ECT technique to study the nature of seed transmission. Later, this technique was used successfully for the identification and detection of BCMV in urad bean, *Runner bean mosaic virus* in runner bean, and *Lettuce mosaic virus* (LMV) in lettuce seeds (Mishra et al. 1967; Agarwal et al. 1979; Ramachandran and Mishra 1987). Besides providing an improvised version of "growing-on" system, culturing embryos on synthetic media permits the virus to attain desired detectable concentration. This modification is not only found very useful for the expression of symptoms of newly emerged leaves but also provides better detection promise for other conventional techniques.

7.2.3 Indicator Plants

Indicator plants are the plants which produce characteristic symptoms having low incubation period after mechanical inoculation. The characteristic symptoms displayed in the form of local or systemic lesions help in the identification of the SBV. Albrechtsen (2006) has provided detailed and elaborated description on the indicator hosts. The most widely employed technique is the direct seed test (DST). This assay includes examination of dry seed and an infectivity assay. In DST,

suspected abnormal seeds are soaked in an aqueous medium followed by soft grinding in a mixer or in a mortar with pestle. The resulted slurry is inoculated onto the indicator plants followed by immediate rinsing of leaves with water. The inoculated seedlings and newly appeared young leaves are observed for symptom expression. The typical virus symptoms in the form of hypersensitive response (local lesions) or systemic reactions (e.g., mosaics, vein clearing, chlorosis, line pattern, stunting, necrosis, etc.) are recorded on the inoculated test seedlings. Using this assay, seed samples either individual or in composite form can be processed with ease for testing virus infectivity.

Sometimes plant viruses (e.g., TMV in tomato seedlings) can be identified by simple inoculation of seed wash onto the indicator hosts. Various other examples of indicator plants include tobacco for *Tobacco rattle virus* (TRV) (Sol and Seinhorst 1961); bean for BCMV (Quantz 1962); pea for *Pea early browning virus* (PEBV) (Van Hoof 1962); *Chenopodium quinoa*, *C. murale*, *C. amaranticolor*, *Phaseolus vulgaris*, and *Vigna sinensis* for nepoviruses (Lister and Murant 1967); *P. vulgaris* var. Scotia; and *N. glutinosa* and *N. tabacum* var. Xanthi, hypersensitive cultivars of bean, tobacco, and cucumber cotyledons for TMV (Phatak 1974; Shmyglya et al. 1984); Bountiful and Top Crop beans for AMV (Frosheiser 1974); *C. quinoa* for LMV (Kimble et al. 1975); tomato and *Solanum berthaultii* for PSTVd (Yang and Hooker 1977; Singh et al. 1991); *C. amaranticolor* and pea cv. 'Perfection' for PSbMV (Hampton et al. 1976); and *Nicotiana tabacum* 'Xanthi NN' for the detection of *Tobamovirus* associated with tomato seeds (Grimault et al. 2012).

To process a large number of seed samples in limited space, detached leaf assay (DLA) has been used. This assay involves incubation of detached leaves onto the moist filter paper in either glass or plastic Petri plates under artificial illumination of ca.1000 lux for 3–4 days. Quantz (1962) successfully demonstrated this assay for the detection and monitoring of BCMV infection in bean (cv. Top Crop) seedlings. Similarly, Phatak (1974) performed DLA for the detection of TMV in tomato seed and successfully recorded TMV symptoms in *N. glutinosa* and *N. tabacum* var. Xanthi nc (Phatak 1974). It is important to mention here that for the plant viruses present in very stumpy concentration in dry seed coats, seed coat has been removed prior to inoculum preparation (Gilmer and Wilks 1967). Thus, the success of this assay largely depends on seed material and the virus strain.

7.3 Imaging Techniques

Imaging techniques like light microscopy (LM) and electron microscopy (EM) provide very promising information on the structural features (e.g., size and shape) of the virus particles and are routinely employed for virus detection where the EM instrumentation is readily available for use. Filamentous and rod-shaped viruses (e.g., potexviruses, potyviruses, and tobamoviruses) can be easily differentiated in negatively stained leaf-dip preparations compared to isometric viruses and other viruses (Walcott 2003). Viruses that occur in low concentrations in plant sap are difficult to visualize unless the virus in the test material is in sufficient

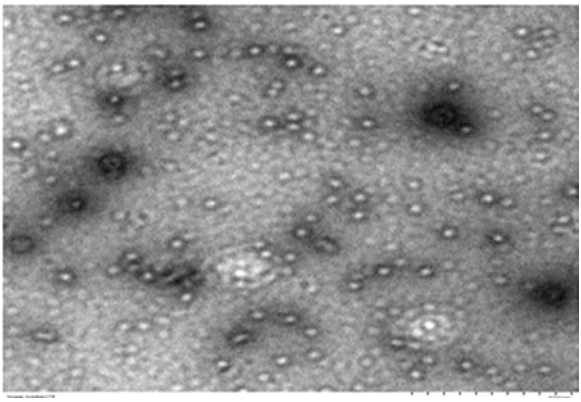

Fig. 7.3 Isometric particles of *Cucumber mosaic virus* observed through IEM

concentration. The efficiency of virus visualization can be enhanced by incorporating serology principle. Immunosorbent electron microscopy (IEM) is a best-quoted example for serology-based improvisation in EM. Kang et al. (2010) detected the presence of isometric particles CMV (30 nm) in summer squash through IEM (Fig. 7.3) As EM is costly and labor-intensive, it cannot be routinely employed for the speedy processing of manifold samples at a time. On the other hand, viruses with elongated particles are simple and easy to identify as the size limit of most groups of viruses is sufficiently divergent from others (Harrison et al. 1971; Francki et al. 1991). Besides this, particle morphology is very useful for preliminary separation of isometric viruses (25–30 nm). The shapes of various isometric viruses include round and knobby (tombusviruses and tymoviruses), round and smooth (cucumoviruses and bromoviruses), angular (nepoviruses, comoviruses, and fabaviruses), ovoid or imperfectly spherical (ilarviruses), etc. (Sastry 2013). The plant sample processing and extraction of virus from seeds differ with host and hence require modifications for removing inhibitors during infected seed processing (Pena and Trujillo 2006). However, sparse information existed on the practical utility of this assay. Phatak (1974) detected BSMV, BCMV, and SMV in the extract of bean plumule, but failed to detect LMV in lettuce seed. In India, EM is also used successfully for the processing of imported germplasm in the form of planting material of numerous crops from various regions of the globe for the detection of intercepted STV (Chalam et al. 2009). However, the application of EM for regular detection is expensive, although, if accessible, it requires less time compared to the germination test for diagnosis of plant viruses.

It is also feasible to detect different types of strains of STV by identifying the shapes of inclusion bodies. Several plant viruses develop unique intracellular inclusions or show large crystalline accretions of virus particles, and their identification by light microscopy (LM) can offer an easy, quick, and comparatively economical way to corroborate viral infection (Edwardson et al. 1993). For example, Garbaczewska et al. (1997) detected *Soil-borne wheat mosaic virus* (SBWMV) particles and inclusion bodies in 3-day-old seedlings of rye (*Secale cereale*). The application of these inclusions for the speedy detection of STV plays an important role

at plant quarantine laboratories where ample diagnostic amenities do not exist for tentative virus detection and diagnosis. Usually, most of these inclusion bodies are observed in the epidermis, hair cells, and cytoplasm of mesophyll and in few instances have been reported from vacuoles, nuclei, and phloem cells. Because of the distinctiveness of inclusion bodies formation as a consequence of virus invasion in plants, mysterious viruses can be recognized to the genus level by observing inclusion bodies with the help of selective stains (Harrison et al. 1971; Matthews 1982). However, these plant virus inclusion technologies require widespread hands-on practice and moreover are unworkable to use alone for the routine identification of STV.

7.4 Serological Viral Detection Assays

Serological assay for the detection of STV is based on the utilization of monoclonal or polyclonal antibodies exclusively attached to a target antigen, permitting the pathogen to be diagnosed by enzymatic conversion of substrates or with the help of fluorescent tags (Kashyap et al. 2016). Generally, the assays based on serology principle are majorly divided into liquid-phase assay (LPA) and solid-phase assay (SPA). In LPA procedure, both antigen and antibody are added in a solution to form a detectable precipitate or agglutination of cells. On the other hand, in SPA procedure, a solid surface such as a microtiter plate or nitrocellulose membrane is used for the reaction of antigen and antibody, which is later visualized by using labeled antibody with either a fluorescent molecule or a radioactive or an enzyme (Webster et al. 2004; Rao and Singh 2008; Koenig et al. 2010). Precipitin assay relies on the production of a visible precipitate when sufficient quantity of virus and specific antibody is in contact with each other (Van Regenmortel 1982). This assay was successfully used by Kumar et al. (1991) for the quick detection of PSbMV in pea (*Pisum sativum*) seeds. On the other hand, in diffusion assays, the diffusion of antigen (either purified virus or virus-infected plant sap) and antibodies in a gel matrix forms a visible precipitin line (Ouchterlony 1962). This assay can be employed to discriminate related but dissimilar strains of a virus or even distinct but serologically matched viruses. However, major demerits of this assay include (i) poor sensitivity in detecting viruses present in low concentration (e.g., luteoviruses and most viruses of woody hosts); (ii) require separation of filamentous or rod-shaped viruses for their simple diffusion through the gel matrix; and (iii) require large amount of antibodies. This assay can detect virus concentrations of 10–25 μg ml^{-1} and also has the potential to confirm the presence of virus even in a single seed or parts of a seed. This method has been widely employed for the routine detection of *Blackeye cowpea mosaic virus* (BlCMV) in cowpea, TMV in tomato seeds, and CMV in French bean (Phatak 1974; Lima and Purcifull 1980; Padma and Chenulu 1985).

In an agglutination assay, the antibody is coated on the surface of an inert carrier particle, and outcome of antigen-antibody reaction can be seen by the naked eye or under a microscope (Koenig et al. 1979; Walkey et al. 1992). This assay is more sensitive than other precipitin tests and can be performed with lower concentration

of reactants (Hughes and Ollennu 1993). This assay is able to detect as low as 1 infected seed in a lot of 100 seeds (Carroll 1979). Moreover, this assay is very useful for the large-scale certification as well as disease resistance screening of potatoes.

Solid-phase tests involve the use of solid surface using labeled antibodies owing to the fact that labeling of antibodies can enhance the sensitivity and provide results of antigen-antibody reactions with a short time of span. Moreover, labeled antibodies are very useful in quantifying the virus in a very little amount of sample (Fateanu 1978). Generally, antibodies are labeled with fluorescent dyes, ferritin, radioactive iodine, and enzymes to confirm the virus in any plant tissue by using autoradiography, fluorescent microscope, and electron microscopy tools (Ball 1974; Andres et al. 1978; Johnson et al. 1978). Using this assay, Alvarez and Campbell (1978) detected SqMV in infected embryos, seedling protoplasts from cotyledons, and microtome sections of dry embryos or seedlings by staining with fluorescein isothiocyanate. The major merit of this assay is that the virus can be detected from 1-week-old cotyledons.

The enzyme-linked immunosorbent assay (ELISA) is another serological method for the identification of viruses based on antibodies and color change in the assay (Fang and Ramasamy 2015). In this method, antigens from the viruses are made to specifically bind with antibodies conjugated to an enzyme. The detection can be visualized based on color changes resulting from the interaction between the substrate and the immobilized enzyme. For instance, detection of seed-transmitted BPMV, CAbMV and PepMoV using ELISA has been reported (Ojuederie et al. 2009; Tsedaley 2015; Sharma et al. 2018). This test has been used to estimate relatively low levels of PepMV in infected seeds (Córdoba-Sellés et al. 2007). From the past three decades, polyclonal antibody-based ELISA are extensively applied for the field detection of plant viruses. Puttaraju et al. (2004) and Nalini et al. (2006) developed polyclonal antibody for the detection of BICMV in cowpea and BCMV in French bean and found it good for seed health testing. Takeuchi et al. (1999) developed direct immunostaining assay (DISA) for the detection of *Tobamoviruses* in pepper seeds. Briefly, in this assay, the seeds provide excellent solid phase in an immunoassay, and resultant stained seeds indicate virus-infected seeds. This assay influences neither the biological detection of viruses nor the viability of seed material (Albrechtsen 2006). Thus, ELISA-based assays can be applicable for the rapid testing of multiple seeds for a single virus using one well per plant sample, or alternatively a single plant can be concurrently evaluated for several viruses on a single plate using different antibodies coated to each well in replicates for better reproducibility. The main disadvantage of the assay is the need of polyclonal or monoclonal antibody sera specific for each virus of interest that does not cross-react with other plant proteins, but cross-absorption with plant sap takes care of this hurdle to some extent.

An enzyme immunoassay (EIA) that has been applied to seed-borne viruses is enzyme-assisted immunoelectroblotting (IEB) (Towbin et al. 1979) and later named as dot-immunobinding assay (DIBA) by Hawkes et al. (1982). Similar assay has been reported in literature by several names like dot-blot immunobinding assay (DIBA) (Berger et al. 1984), immunoblot assay (Powell 1984), enzyme-linked immunoblot assay (EIBA) (Wang et al. 1985), NC-ELISA (Bode et al. 1984), and

NCM-ELISA (Smith and Banttari 1984). However, this technique is popularly referred to as dot-immunobinding assay (DIBA) or dot-ELISA. Principally, DIBA is similar to ELISA except that antigen or antibody is bound to nitrocellulose membrane instead of polystyrene plate and that the product of the enzyme reaction is insoluble. This assay has been applied for the detection of BCMV in bean (Lange et al. 1989), PSbMV in peas (Ali and Randles 1997), CGMMV in cucurbitaceous crops (Shang et al. 2011), and *Broad bean true mosaic comovirus* (BBTMV) in broad beans (Makkouk and Kumari 1996; Khatab Eman et al. 2012). The ability to reliably detect potato viruses by DBIA with an equal sensitivity to that of DAS-ELISA was also reported by Palma et al. (2013). Several trials showed that multiplex tissue print immunoassay (TPIA) and dot-blot immunoassay (DBIA) might represent fast, practical, and sensitive alternatives for the detection of *Potato leaf roll virus* (PLRV), *Potato virus S* (PVS), *Potato virus X* (PVX), and *Potato virus Y* (PVY) from non-germinating potato tubers (Samsatly et al. 2014). The major merit of this assay are (i) rapid, simple, and economical; (ii) requires only a single crude-specific antiserum for each test virus and also a single generally applicable enzyme conjugate; (iii) highly sensitive and detects as low as 50–100 picogram of antigen; (iv) cost of nitrocellulose is less than that of plastic supports; and (v) part of the seed can be used for testing and the remaining for sowing. However, a major demerit of this assay is the huge requirement of concentrated (1 mg ml^{-1}) antiserum; however, it can also be reused over a period of 6 months to test the plant samples (Abdullahi et al. 2001).

Tissue blot immunobinding assay (TBIA) is similar to dot-blot immunoassay but involves tissue in printing on nitrocellulose membrane (NCM). This assay does not involve disruption of tissue or extraction of antigen from the seed sample. The fresh material or imbibed seed material can be used as an imprint tissue on NCM (Lin et al. 1990). Khatab Eman et al. (2012) have used this assay to detect BBTMV in faba beans. This assay was sensitive enough to detect the virus in all parts of the plant and at all growth stages. It is suggested that the test is useful for detecting seed-transmitted viruses after seed germination and is more practical than ELISA. This test can be completed in less than 4 h without sacrificing sensitivity and is cheap and does not require sophisticated facilities. This assay is easily applicable to field sampling as tissue printings can be made in the field without the need to collect samples for sap extraction in the laboratory.

Disperse dye immunoassay (DIA) is a solid-phase immunoassay in which enzyme conjugate is substituted as a consequence of rapid mixing of dye molecules with an organic solvent, for instance, dimethyl sulfoxide (DMSO). For color development, DMSO is added in the sample plates. After shaking of plates for a minute, color intensity is recorded at 540 nm (Gribnau et al. 1982). This assay was used by Van Vuurde and Maat (1985) to test seeds of lettuce and pea for the presence of LMV and PEBV, respectively. The major merits of the assay include (i) simple and cheap procedure for conjugate preparation; (ii) no need of substrate incubation step; and (iii) simultaneous detection of two distinct types of antigens (Gribnau et al. 1983). However, this assay is inappropriate for crude plant extracts. Moreover, in comparison to ELISA, it requires a high amount of IgG for the preparation of dye solution conjugate. Over all, DIA is equivalent to ELISA in terms of sensitivity and offers a good option for indexing of seed material of different crops at a large scale.

Voltametric enzyme immunoassay (VEA) is also reported as a modification of ELISA, where rather than a color, change in electrical conductivity of the substrate is monitored, when an enzyme linked with a secondary antibody. This method offers a high magnitude of sensitivity in comparison to ELISA. It was successfully reported for the detection of *Cucumber mosaic virus* (Sun et al. 2001).

7.5 Molecular Viral Detection Assay

Molecular assay based on polymerase chain reaction (PCR) has plentiful assenting features, including specificity, sensitivity, rapidity, and simple interpretation, which makes it convenient for the detection of SBV in agricultural crops. Suspected infected seed materials have been examined in PCR by using specific primer pairs or DNA barcodes (Kashyap et al. 2017a). The specific primer sets for different SBV (Table 7.3) enable PCR to detect specific viruses. Several modifications of the basic PCR technique (e.g., RT-PCR, nested PCR, multiplex PCR, IC-PCR, real-time PCR, etc.) have been done to enhance the specificity and sensitivity with automation for the rapid detection of SBV which is discussed in the following sections. Besides all these techniques, LAMP technique has also been used now-a-days to detect viruses which do not involve the use of PCR machine.

7.5.1 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

From the last three decades, PCR and reverse transcription polymerase chain reaction (RT-PCR) are recognized as excellent techniques for the amplification of the genomic DNA or complementary DNA (cDNA) in a reasonably short time and, moreover, cutting several time-consuming steps of conventional gene cloning methodologies. These techniques are very useful in detecting the virus at picogram level in infected planting seed material and also for the verification of appropriate DNA insertion in the transgenic plants. Sambade et al. (2000) for the first time reported single-step RT-PCR procedure for the specific amplification of cDNA from the plant virus containing RNA as genetic material. At present, more than 200 viruses and viroids have been reported by using RT-PCR in different types of vegetable and horticultural crops. This technique was successfully applied for the detection of peanut seed-transmitted potyviruses and cucumoviruses (Dietzgen et al. 2001); *Cowpea aphid-borne mosaic virus* (Gillaspie et al. 2001), *Broad bean stain comovirus* (BBSV), and CABMV in faba bean and cowpea plants (El-Kewey et al. 2007); CMV and ZYMV in *Cucurbita pepo* (Tobias et al. 2008); BlCMV in cowpea (Udayashankar et al. 2009); and PSTVd in potato (Shamloul et al. 1997). Recently, Gumus and Paylan (2013) applied RT-PCR for the detection of *Cucumber mosaic cucumovirus* (CMV), *Tomato mosaic tobamovirus* (ToMV), *Alfalfa mosaic alphavirus* (AMV), LMV, CGMMV, TBSV, TMV, and TBRV in seeds of cucumber, tomato, pepper, and lettuce. The RT-PCR assay is found to be five times more sensitive than standard ELISA.

Table 7.3 Primers available for detection of SBV in different crops by PCR

S. no.	Virus	Primers (5′-3′)	Thermal profile	Product size (bp)	Reference
1	*AMV*	F:GT GGT GGG AAA GCT GGT AAA R:CAC CCA GTG GAG GTC AGC ATT	1X (94 °C 2 min) 35X (94 °C/30 s, 54 °C/30 s, 72 °C/30 s) 1X (72 °C 10 min)	700	Martínez-Priego et al. (2004)
2	*CABMV*	F: CGCTCAAACCCATTGTAGAA R:TATTGCTTCCCTTGCTCTTTC	1X (94 °C/2 min) 35 X (94 °C/30 s, 50 °C/30 s; 72 °C/60 s) 1X (72 °C/10 min)	221	Gillaspsie et al. (2001)
3	*CGGMMV*	F:GTT TCG CCT CAA AAT TCC R:TCT AAA TAT GAC AAG TCG C	1X (95 °C 5 min) 35X (94 °C/30 s, 55 °C/30 s, 72 °C/90 s) 1X (72 °C 10 min)	359	Moreno et al. (2004)
4	*CGMMV*	F: CCGAATTCATGGCTTACAATCCGA TCAC R:TTGCATGCT GGGCCCCTACCC GGG GAA AG	1X (94 °C/3 min) 35X (94 °C/30s, 53 °C/45S, 72 °C/1 min) 1X (72 °C/7 min)	700	Hongyun et al. (2008)
5	*CMV*	F:GAGTCGAGTCATGGACAAATC R:GGCTGCAGTGGTCTCCTT	1X (94 °C/3 min) 30–35X (94 °C/3 s, 60 °C/3S, 72 °C/20s) 1X (72 °C/7 min)	950	Ali and Kobayashi (2010)
6	*CMV*	F:CGCCCT GCAGTGGTCTCCTTTTGGAG R:ACT CCA ACT GGC TCG TAT GG	1X (92 °C/2 min) 30X (94 °C/60 s, 58 °C/20 s, 75 °C/60s) 1X (75 °C/5 min)	600	Nakazono-Nagaoka et al. (2005)
7	*CPMoV*	F:AAC AAC GGA GAT GAC TGT GT R:TAC GCT TGC TGT ATG GGT AT R:TTG AAG AGA GGG AGG AGA AG	1X (94 °C/2 min) 25 X (94 °C/1 min, 52.5 °C/2 min, 72 °C/3 min) 1X (72 °C/10 min)	–	Gillaspsie et al. (1999)
8	*CSSV*	F: AACCTTGAGTACCTTGACCT R: TCATTGACCAACCCACTGGTCAAG	1X (95 °C/15 min) 35X (94 °C/30 s, 56 °C/90 s, 72 °C/60 s) 1X (60 °C/30 min)	–	Quainoo et al. (2008)

(continued)

Table 7.3 (continued)

S. no.	Virus	Primers (5′-3′)	Thermal profile	Product size (bp)	Reference
9	*DMV*	F: ATGGATCGTAAAGATT R:CTGTTTTTCTGTGTTTCTACTGG	1X (94 °C/3 min) 50X (94 °C/30 s, 50 °C/20 s, 72 °C/1 min per 1000 bp product) 1X (72 °C/7 min)	900	Pahalawatta et al. (2007)
10	*DMV*	F:TGCATAAAATGAGTTCTATC R:TGAACTTGTTCATCATTATC	1X (94 °C/3 min) 50Xng of 30 s at 94 °C, 20 s at 58, and a 1 min extension per 1000 bp product at 72 °C, followed by a final extension for 7 min at 72 °C.	480	Pahalawatta et al. (2007)
11	*DMV*	F- ATGGAAGAAATTAAGGCGT R- TTGTCTTCATCCATAAAGCAG	3 min at 94 °C for initial denaturation; 50 cycles each consisting of 30 s at 94 °C, 20 s at 60, and a 1 min extension per 1000 bp product at 72 °C, followed by a final extension for 7 min at 72 °C.	1280	Pahalawatta et al. (2007)
12	LMV	F:AAG GCA GTA AAA CTG ATG R:TTT ATA CTA CAG TCT TTA	1X (94 °C 1 min) 35X (94 °C/60 s, 55 °C/120 s, 72 °C/120 s) 1X (72 °C 10 min)	800	Zerbini et al. (1995)
13	*PeMoV*	F: TCA ACG GAA ATT GGA CCA TGA T R: GTT CCG ACG TTA CCA TCA AGA CCA	1X (37 °C/1 h) 1X (94 °C/2 min) 35X (94 °C/30 s, 50°/30 s, 72 °C/60 s) 1X (72 °C/10 min)	339	Gillapsie et al. (2000)
14	*PStV*	F: GGCGAGTATGAAATA GAT R: GGTGGTAAA ACC ACA CTG	1X (37 °C/1 h) 1X (94 °C/2 min) 35X (94 °C/30 s, 50 °C/30 s, 72 °C/60 s) 1X (72 °C/10 min)	611	Gillapsie et al. (2000)
15	*TBRV*	F: ATGGGAGAAGTGCTGG R: AATCTTTTTGTGTCCAAC	1X (95 °C/5 min) 35X (94 °C/60 s, 54 °C/60 s, 72 °C/120 s) 1X (72 °C/7 min)	330	Le Gall et al. (1995)

16	*TBSV*	F: GCA AAC TGT GCA GAT GAC TGT G R: CAC AGT CAT CTG CAC AGT TTG C	1X (95 °C/5 min) 35X (94 °C/60 s, 60 °C/60 s, 72 °C/120 s) 1X (72 °C/7 min)	620	Boonrod et al. (2004)
17	*TMV*	F:GACCTGACAAAAATGGAGAAGATCT R: GAA AGC GGA CAGAAA CCC GCT G	1X (95 °C/5 min) 40X (94 °C/30 s, 50 °C/60 s, 72 °C/60 s) 1X (72 °C/7 min)	422	Jacobi et al. (1998)
18	*ToMV*	F:CTC CAT CGT TCACAC TCG TTA CT R: GATCTGTCAAAG TCT GAGAAA CTT C	1X (94 °C/2 min) 35X (94 °C/30 s, 62 °C/45 s, 72 °C/60 s) 1X (72 °C/5 min)	508	Jacobi et al. (1998)
19	*WSMV*	F:5′GGG CTTGATGTRACAGAGG R:TCACATCATCTGCATCATGACGTG	1X (94 °C/3 min) 30X (94 °C/30s, 55 °C/30s, 72 °C/1 min) 1X (72 °C/7 min)	493	Jones et al. (2005)
20	*ZYMV*	F: AAGTGAATTGGCACGCTA R: CGGTAAATATTAGAATTACGTCG	1X (98 °C/1 min) 2X (98 °C/10 s, 64 °C/20 s (minus 1 °C every cycle), 72 °C/40 s) 31X (98 °C/10 s, 62 °C/20 s, 72 °C/40 s) 1X (72 °C/5 min)	–	Simmons et al. (2011)

7.5.2 Multiplex Polymerase Chain Reaction (M-PCR)

Multiplex PCR (M-PCR) reported by Chamberlain et al. (1988) had tremendously transformed the field of the molecular diagnostics of plant viruses by PCR. This technique is very easy to use and successfully reported for the simultaneous detection of several DNA and RNA viruses of agricultural crops in a single tube (Nassuth et al. 2000). Moreover, it was found very useful for the identification and detection of plant viruses that exist as multiple or cause mixed contamination in the same seed lot. Principally, M-PCR amplifies several sets of oligonucleotides targeting the genomic region of various plant viruses belonging to the same genus and same species or from taxonomically different groups in a single PCR reaction. Thus, this assay provides the advantage of expediency, rapidity, and cost saving by saving reagent cost against individual PCR reaction (Deb and Anderson 2008). Ge et al. (2013) developed multiplex RT-PCR method for concurrent detection and discrimination of PVS, ToMV, PepMV, and PVM in pepino. Kwon et al. (2014) developed dual primer sets for devising two different multiplex PCR assays for the simultaneous diagnosis of seven unique and distinct viruses of cucurbits. The first primer set-based M-PCR assay was reported for the successful detection of *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), and ZYMV, whereas the another primer set was found suitable for rapid detection of *Kyuri green mottle mosaic virus* (KGMMV), CGMMV, *Cucumber fruit mottle mosaic virus* (CFMMV), and *Zucchini green mottle mosaic virus* (ZGMMV). Thus, the application of multiple primer sets designed against various target sequences in the same reaction saves time and lessens running cost of M-PCR assays employed for the virus detection (Yadav and Khurana 2016).

7.5.3 Immunocapture Polymerase Chain Reaction (IC-PCR)

Immunocapture polymerase chain reaction (IC-PCR) is developed by combining the merits of serology to capture virus particles by antibodies and advantages of PCR to amplify a target gene using virus-specific primers. The assay involves the adsorption of the virus particles on antibody-coated microtiter plate or small-size tube (2 ml or less volume) followed by heating in the presence of a nonionic surfactant (e.g., Triton X-100) and amplification of target nucleic acid by executing RT-PCR assay. In this method, the antibody helps in the capturing of viruses which ultimately assist in the separation and purification of the virions from the infected plant samples, even when the concentration of virus is too low or when extracted nucleic acid contains PCR inhibitor compounds limiting the successful amplification of the desired product. Hence, this method is very useful for the field diagnosis of various viruses present in low concentration (Le Provost et al. 2006). IC-PCR has been successfully demonstrated for the sensitive and specific diagnosis of TSWV, *Pepper mild mottle virus* (PMMV), and CMV along with CMV satellite RNA (Narayanasamy 2011). The principal benefit of this highly specific method is the amalgamation of serological efficacy and nucleic acid amplification, which provide

special advantage in removing PCR hindrances posed by contaminated RNA of viruses such as PVY and tobamoviruses (Gawande et al. 2011).

7.5.4 Duplex Reverse Transcription Polymerase Chain Reaction (Duplex RT-PCR)

Duplex reverse transcription polymerase chain reaction (RT-PCR) assay was developed by Dietzgen et al. (2001) for confirming the presence of seed-borne cucumoviruses (CMV and PSV) and potyviruses (PeMoV and PStV) in peanut. Briefly, this method involves several steps and includes i) binding of the virus particles extracted from crude plant sample to the PCR tube; ii) removal of unbound particle and debris by washing; iii) lysis of viral particles in a medium; and iv) PCR for desired product amplification. This method is very simple and inexpensive. However, the rate of success and level of detection of this assay are reported to be lower than IC-PCR for detecting virus in plant hosts containing enormous quantity of polyphenols.

7.5.5 Nested PCR

Nested PCR assay is developed to overcome the limitations of successful amplification of low levels of the target virus in seeds. Mancini et al. (2016) mentioned that nested PCR is able to detect desired DNA at several-fold lower concentration than conventional PCR. In this method, the amplified product of the first few PCR cycles (using a first primer set) is employed as a base template for the second set of nested primers designed for the amplification of the target amplicon. This method was developed to improve the specificity of virus detection in different plant samples and also to amplify the copies of viral nucleic acid when plant samples contain vary low concentration of virus. This method was successfully demonstrated for the detection of *Beet necrotic yellow vein virus* in sugar beet crop (Morris et al. 2001).

7.5.6 Real-Time PCR

Traditional PCR is reported to be highly sensitive, specific, and rapid for the amplification of target DNA. However, the major disadvantage of this assay is its qualitative nature. In contrast, real-time PCR is capable to produce a specific fluorescent signal detected by fluorometer to offer real-time analysis of reaction kinetics resulting in quantification of specific DNA target (Ward et al. 2004; Kashyap et al. 2011; Kumar et al. 2013; Singh et al. 2014; Mirmajlessi et al. 2015). With minimum sample processing, real-time PCR lessen the probability of false positives, generally, which occurred due to cross-contamination of chemicals used in the reaction mixture (Tomlinson et al. 2005). This method is found to be highly time-saving in comparison to other PCR-based method and moreover offers high sensitivity of detection of viral DNA up to the level of femtograms (Chilvers et al. 2007). It has been

observed that when employed for virus detection in planting material and seeds, quantitative real-time PCR assays, with the use of SYBR Green dye and TaqMan-labeled probes, reflected a high level of sensitivity in the detection and quantification of SBV. For instance, real-time PCR based on TaqMan chemistry was used to detect as low as 500 fg total RNA of *Tomato spotted wilt virus* (TSWV) in infected tomato plants and observed more sensitive (tenfold) than the traditional RT-PCR (Roberts et al. 2000). Boonham et al. (2000) reported a multiplex real-time PCR procedure to detect as low as single particle of *Tobacco rattle virus* (TRV) and *Potato mop-top virus* (PMTV) in potato tubers. Similarly, Klerks et al. (2001) employed real-time PCR to detect individual and mixed infections of PLRV and PVY in potato tubers. A two-step multiplex real-time PCR assay was also reported to detect PVY, PLRV, PVX, and PVA from the sap of potato tubers (Agindotan et al. 2007). Fox et al. (2005) documented that real-time PCR is a more reliable assay than ELISA for the detection of PVY in potato tubers. Besides this, real-time PCR assay also reported for the detection of seed-transmissible *Tomato yellow leaf curl virus* (TYLCV-IL) in tomatoes (Kil et al. 2016) and PSTVd in potato (Boonham et al. 2004). Recently, efforts have been made to develop multiplex real-time PCR assay by employing multiple primers (together with probes in the TaqMan assay) in the same reaction, which ultimately lead to cost and labor minimization. Furthermore, a high-throughput, real-time multiplex, single-tube RT-PCR assay has been reported for the simultaneous detection of PLRV, PVS, and PVX in potato leaves and tubers and TSWV in potato tubers (Mortimer-Jones et al. 2009). It is likely that more real-time PCR seed assays will be developed as the technology becomes more affordable. However, to maintain the desired level of specificity, sensitivity, and analogous amplification efficiency of different viruses in real-time PCR assays, it is essential to select the suitable target DNA fragment to design the best primers and probes.

7.5.7 Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a powerful and novel nucleic acid amplification method that amplifies target DNA with high specificity, efficiency, and rapidity under isothermal conditions. This assay utilizes two pairs of primers that target six regions of a gene sequence and trigger specific and isothermal amplification of the target sequence, thereby purging the general requirement of PCR (Kashyap et al. 2014). Lenarčič et al. (2013) reported a single-tube, real-time, reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection of *Potato spindle tuber viroid* (PSTVd) in potato. In a similar fashion, RT-LAMP assays for TYLCV and *Melon yellow spot virus* (MYSV) use toothpicks for sample template acquisition (Shiro et al. 2005; Takeuchi et al. 2006). Besides this, LAMP assay has been developed and successfully used to detect several viruses such as CMV, PVY, TMV, and *Tomato necrotic stunt virus* (ToNStV) (Zhao et al. 2012; Li and Ling 2014).

7.5.8 Microarrays (DNA Chip)

Microarrays or DNA chips represent another nucleic acid-based detection assay that can be used to confirm the presence of viral infection in seeds. This technique relies on the unique ability of nucleic acid molecules to hybridize specifically with molecules with complementary sequences. With microarray technology, oligonucleotide probes are attached to small (~1 cm^2) glass or silica-based surfaces (chips). The power of this method lies in the fact that hundreds to thousands of oligonucleotides can be attached to specific locations on each chip. These oligonucleotides are generally complementary to DNA sequences that are unique to STV and, hence, can be used to detect viruses in seed lots. Microarray-based detection of PVY, PVA, PVX, and PVS in single infection or mixed infection in potato tubers has been reported by Boonham et al. (2003). A plant virus cDNA chip was devised for the detection and determination of cucurbit-infecting *Tobamovirus* (Lee et al. 2003). Good detection selectivity was attained for two potato viruses (PVS and PLRV) by Bystricka et al. (2003). Further, the same group has developed a microchip for detection of PVA, PVS, PVM, PVX, PVY, and PLRV in both single and mixed infections in potato tubers (Bystricka et al. 2005). Deyong and associates (2005) described microarray application in the detection and differentiation of 14 different isolates of CMV belonging to different serogroups and subgroups. Macroarray detection of potato-infecting viruses and viroids has been reported by several workers (Agindotan and Perry 2008; Maoka et al. 2010). Engel et al. (2010) developed an array of 570 unique probes comprised of highly conserved and species-specific regions of 44 plant viral genomes. From the above-mentioned advancements, it can be concluded that microarray-based detection of plant viruses will be of great utility to quarantine departments for the purpose of biosecurity from important viral diseases of seed-borne nature. However, it is foreseen that microarrays that drive diagnostic platform will be more widely employed for regular seed testing in the near future.

7.5.9 Next-Generation Sequencing (NGS)

Advances in next-generation sequencing (NGS) technologies have offered a promising alternative for the detection of SBV. Using small RNA (sRNA) library construction and deep sequencing approach, NGS is able to recognize both known and novel virus species and de novo assemble virus and virus-like genomes. Li and associates (2012) used sRNA deep sequencing and detected PSTVd, *Pepino mosaic virus* (PepMV), and an unknown *Potyvirus* species in the infected tomato samples (Li et al. 2012). Several computational algorithms have been developed and employed for the quantitative detection of the viroids using deep sequencing (Bolduc et al. 2010) or in a homology-independent manner (Wu et al. 2012). However, NGS is very cumbersome and computation-intensive and, moreover, highly expensive than the normally used conventional PCR or ELISA protocols.

7.6 Nanosensor-Based Viral Diagnostic Assay

Nanosensor with immobilized bioreceptor probe designed specifically for targeting the analyte molecules is defined as nanobiosensors. They provide the benefits of being small, portable, sensitive with real-time monitoring, precise, quantitative, reliable, accurate, stable, reproducible, and robust to detect and report potential and complex disease problems (Kashyap et al. 2016; Kashyap et al. 2019). Recent reports indicated that nanowire array can be combined with multiple biorecognition for developing nanobiosensors. Principally, nanowire coated with any biological molecules (e.g., DNA molecules, polypeptides, fibrin proteins, bacteriophages, etc.) is used as a sensing component in nanowire biosensors (Kashyap et al. 2017b). This may be due to the fact that the surface properties of nanowires can be easily modified and hence can be coated with any kinds of a chemical or biological molecular recognition unit and resulting in analyte-independent nanowire functioning. It has been observed that the nanomaterials transduce the chemical binding event on the nanowire surface and as a result change in conductance of the nanowire can be monitored in an extremely sensitive, real-time, and quantitative fashion. This type of nanowires is very useful for high-throughput diagnosis and screening of viruses in infected plant samples. For instance, Ariffin and associates (2014) employed nanowire as a biosensor transducer for the detection of PRSV and CMV in planting materials. The results of their study clearly highlighted that the nanowire acts as an excellent material for fabricating nanoscale biosensors and will be very useful for fabricating remote-controlled nanobiosensors with potential applications in disease diagnostics, plant seed health testing, and environmental monitoring. Polypyrrole (PPy) nanoribbon modified chemiresistive sensors are another type of nanomaterials used in nanobiosensor developments and generally synthesized by employing lithographically patterned nanowire electrode position (LPNE) technique. James (2013) demonstrated the application of this type of fabricated biosensor for the rapid detection of CMV and achieved sensitivity in detection limit of 10 ng mL^{-1} (James 2013). Moreover, this antibody-based biosensor is found to be approximately two orders of magnitude higher than conventional ELISA technique (Perdikaris et al. 2011). Recently, a portable cell biosensor system for the detection of PVY, CMV, and TRV was reported by Kumar et al. (2015). The biosensor was fabricated by immobilizing the vero cells carrying virus-specific antibodies on their membranes. These research advancements will be very useful for the development of portable plant virus detection platform for rapid and sensitive seed health testing as well as for field application in the near future.

7.7 Conclusion

Rapid and sensitive detection of plant viruses is becoming more challenging with the globalization of trade, particularly plant seeds. Plant seeds must be healthy and free from viruses before planting them in the field for which highly sensitive, user-friendly, cost-effective, and specific detection kits are of utmost significance. Presently,

techniques for assessing propagation materials and other field seed samples for the occurrence of specific viruses include biological indexing, electron microscopy, serology-based procedures, PCR, microarray-based detection system, etc. PCR-based assay holds great potential for virus detection in seeds of various crops, as it embodies many of the key characteristics including specificity, sensitivity rapidity, ease of implementation and interpretation, and applicability. New assays like LAMP emerged as a user-friendly, rapid, lucrative, and highly precise technique which could be used even without PCR instrumentation, and it is feasible to utilize this technique for on-site field detection of SBV. Extensive adoptability of NGS technology may take time, although it is a very good tool for the rapid indexing of precious seed germplasm and invention of new viruses without any preceding knowledge. Unfortunately, at present this technology is limited to few laboratories only. Array-based technology is another alternative for the precise and reliable detection of a wide range of STV. However, still several challenges remain in the development of such technologies that are cost-effective and easy to use in the field detection and screening of suspected seed material and also inexpensive to the farmers.

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Detection and Diagnosis of Seed-Borne and Seed-Associated Nematodes

8

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Abstract

Seed is a very good carrier of phytonematodes, especially for long distance dissemination. This transmission occurs either directly through seed infection or via seed contamination. Although in low rate, this transmission becomes a serious concern when trans-boundary movement of invasive alien nematode species flares up in epiphytotic proportion. Hence, detection and diagnosis of seed-borne and seed-associated nematode are significant for their protection. So far, various conventional methods are mostly employed for detection, but advancement of modern approaches, viz. serological or molecular techniques, speeds up the process with more accurate detection. Their robustness and specificity with greater resolution help in identification and discrimination of different species of seed-borne phytonematodes, thus strengthening phytosanitary programme and ensuring low risk in world trade. It also helps in pest risk analysis (PRA) of any pests having quarantine importance and development of national standards for import of seed and planting materials. Besides these, for the identification of pest-free area, production of certified seeds, and promotion of export quality raw plant products, detection of seed and seed-associated nematodes is very essential. Hence, detection is always considered as primary step for crop protection.

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8.1 Introduction

Seed-borne nematodes are one of the most important biotic constraints in the crop production worldwide. Seed and planting materials are the basic units of production for the world's food crops. In recent years, seed has become an international commodity used to exchange germplasm and food grains around the world, which accounts more than ever for the movement of seed-associated nematodes across vast distances, natural barriers, and political borders (Gitaitis and Walcott 2007). They are responsible for both pre- and post-emergence effects on grains, seedling vigour, and also variation in plant morphology, viz. dicolouration, shrivelling and plant vigour (Van Du et al. 2001; Rajput et al. 2005; Niaz and Dawar 2009), and ultimately reduced the yield. Farmers usually blame unfavourable soil or climatic conditions, but if the nematode associated with seed could be managed, yield can be dramatically increased. This infected seed material not only produces harmful toxins but also results in the distribution of nematode problems to new areas, introduction of new strains, or physiologic races of these nematodes along with exchange of germplasm from other countries.

Nematode diseases associated with seed usually go unnoticed as infected plants are rarely killed. They cause direct damage to the seed which may be internal or may occur as seed infestation. Nematodes are migratory or sedentary parasites which evolve from ectoparasitic and endoparasitic associations with plants and are found in rhizospheric soil around the root. Generally, they are host-specific, but most are polyphagous and invade plant hosts, multiply quickly to have several generations per year, and have easy ways for spread and dispersal.

Seed infestation by nematodes does not produce any specific symptoms on seed, but in those that occur as endoparasites, the floral structure may be modified into a seed gall (*Anguina* spp.), may produce symptoms such as discolouration of testa in groundnut (*Ditylenchus destructor*), or may be symptomless as in paddy kernels infected by *Aphelenchoides besseyi*. Neergaard (1979) listed five genera of nematodes which are transmitted by seed, viz. *Anguina, Aphelenchoides, Ditylenchus, Heterodera,* and *Rhadinaphelenchus.* Among these genera, *Heterodera* and *Rhadinaphelenchus* occur as endoparasites in roots, stems, and leaves of the associated plants.

The genera that cause internal infection of seeds, *Ditylenchus, Aphelenchoides,* and *Pratylenchus* are soil- as well as seed-borne. They are facultative endoparasites and produce most stages in the soil. Usually, the pre-adult stage is causing more infection than other stages, except egg and first-stage larvae. There are several examples in which close association of nematodes and seed tissues can be recognized, e.g. *Ditylenchus dipsaci* in lucerne, clover, onion, and beans, *Pratylenchus brachyurus* in groundnut, *Aphelenchoides besseyi* in rice, *Heterodera* in bean, and sugarbeet and *Rhadinaphelenchus* in coconut. Among the seed-borne nematodes, *Anguina* in wheat and rye is the mostly focused in research due to its marked adaptation to parasitism. This is obligate parasite and normally completes its development only after invading the inflorescence and forming galls. Infective second-stage larvae that remain in seed galls come out from the galls released in soil and migrate to new plants. These become associated with the host seedlings on which they feed ectoparasitically until they invade the floral parts.

Table 8.1 List of major genera of nematodes associated with seed

S. No.	Nematodes	Part of seed infected	Host plant
1.	*Anguina tritici*	Seed galls	*Triticum aestivum, Avena sativa, Secale cereale*
2.(a)	*Ditylenchus destructor*	Hull, seed coat, and surface	*Arachis hypogaea*
			Solanum tuberosum
2.(b)	*Ditylenchus dipsaci*	Bulbs, tubers, and rhizomes	*Allium cepa*
2.(c)	*Ditylenchus angustus*	Seed	*Oryza sativa*
3.	*Aphelenchoides besseyi*	Beneath hull	*Oryza sativa*
4.	*Pratylenchus brachyurus*	Hull	*Arachis hypogaea*
5.	*Bursaphelenchus* (*Rhadinaphelenchus*) *cocophilus*	Seeds, seedlings, and nut husk	*Cocos nucifera*
			Phoenix dactylifera

Phytopathogenic nematodes have a well-developed sensory and behavioural system that enables them to locate and attack specific parts of the plants. Dropkin (1969, 1976) has given an account of the process of infection by plant-parasitic nematodes and the various cellular responses. Species of *Anguina* stimulate gall formation in flower parts of grasses. During gall formation, hypertrophy and hyperplasia of the parenchyma cells of the pericarp take place, while the central cavity harbours the nematodes and no syncytia (giant cells) develop in tissues attacked by nematodes.

There are several nematode species causing seed infection, but the major seed-borne or associated nematodes belong to the genera *Anguina*, *Ditylenchus*, *Aphelenchoides*, *Pratylenchus,* and *Bursaphelenchus* (*Rhadinaphelenchus*) *cocophilus*. Some groups of nematodes show host specificity and these are supposed to have co-evolved along with their hosts. Hence, sometimes it becomes important to have knowledge of the host species and the species of nematode. There is difference in portion of the seed infected, but in general their mechanism and adaption are same belonging to associated genera (Table 8.1).

8.2 Symptoms, Penetration, and Survivability of Seed-Borne Nematode

8.2.1 *Anguina Tritici*

Anguina tritici was first time discovered in England in 1743 and was the first plant-parasitic nematode to be recognized (Ferris 2013). It was primarily found in wheat but to some extent also found in rye (Peral 2015). *A. tritici* is found with the seed galls (Fig. 8.1) where all the stages (egg, juvenile, female, and male) are found, which are protected from harsh environmental conditions. This nematode also survives on the soil surface either inside or outside of galls and in dry seed stage. Second-stage juveniles J_2, which is the infective juvenile stage, are capable of entering a cryptobiotic stage (anhydrobiosis) for its survival during dry conditions in drying galls (Bird and

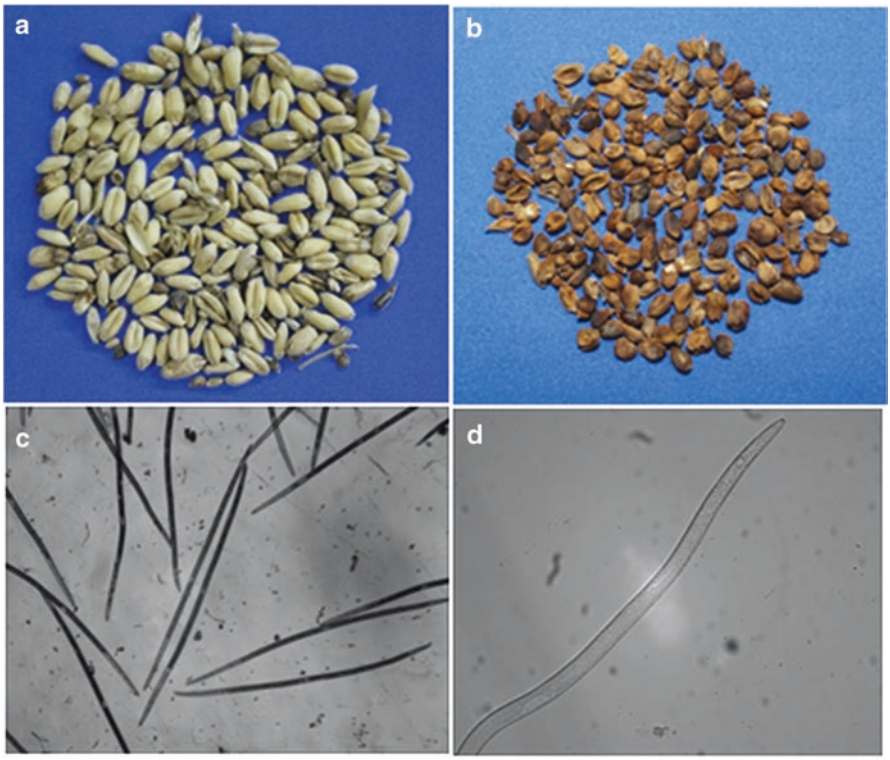


Fig. 8.1 Wheat seed-borne nematode (*Anguina tritici*) (**a**) Infected wheat seed (**b**) Wheat galls (**c** and **d**) Second juvenile stage (J_2) of *Anguina tritici.* (Bar representing 10 μ)

Buttrose 1974), where it can survive for several decades and can survive up to 40 years in storage conditions (Bridge and Starr 2007). When galls become wet and absorb water and rehydrated, juveniles become active (Fielding 1951).

Infective juvenile stage J_2 comes out from the seed galls on the soil surface and migrates to new plants between leaf sheaths and feed as an ectoparasite between compacted leaves until flower primordia comes out (Ferris 2013). Then, they penetrate the developing flower ovaries at the time of initiation of flower bud.

After penetration, J_2s, instead of seed formation, stimulate formation of galls. These galls can be developed from undifferentiated flower ovaries, stamen tissues, and other tissues (Ferris 2013). When the second-stage juveniles mature, each gall has nearly up to 80 adults in a sex ratio of 1:1. Adult female produces up to 2000 eggs, which after hatching progress to J_2s and remain confined within the galls as the survival stage and perpetuate plant infection. Dry galls are harvested along with developed seeds which contain thousands of J_2s (Ferris 2013).

When *A. tritici* feed ectoparasitically, it causes leaf rolling, curling, and spiralling (Anwar et al. 2001). Severe infection of young plants results in stunted growth

with distorted, misshapen stems and leaves. The ear or inflorescence either may be absent or, if present, it is wider and shorter (Bridge and Starr 2007). Infected plants mature slowly and produce smaller seed heads. Infected wheat heads are reduced with glumes protruding to view the galls. Infected developing ovaries are transformed into galls, which are light brown to nearly black in colour. Galls appear darker, shorter, and thicker than healthy seeds. Young galls are short, thick, smooth and light to dark green, turning brown to black with age. Galls contain a white mass of dry nematodes in an anhydrobiotic state which becomes powdery after crushing (Bridge and Starr 2007). Severe infestation of *Anguina* may cause yield losses up to 50% (Ferris 2013).

8.2.2 *Aphelenchoides besseyi*

Aphelenchoides besseyi is also an important seed-borne nematode where primary means of disease transmission in rice is by seed originating from infected plants. Nematodes survive in a state of anabiosis in dried seed, mainly on the inner surface of hulls, but also on the kernels. Nematode-infested plants show white-tip or whip-like malformation on the top third of the leaf blade in flowering tillers, chaffiness, and abnormal elongation of glumes in some of spikelets (Todd and Atkins 1958; Rao et al. 1970). Infected plants show reduced vigour, height and weight of spikelets, and number of grains. In case of severe infestation, abnormal elongation of the panicles and chaffiness or scattered chaffiness in the florets is visible (Prasad et al. 2007). The nematode becomes active at the germination of the seed and starts feeding ectoparasitically on the leaf primordia.

The nematodes feed and multiply in the leaf whorls and climb up to the booting panicle through a fine film of moisture on the surface. Nematodes invade the florets through the tunnel below the apiculus where lemma and palea remain open. As the grain ripens, the nematodes become quiescent in dried tissues of panicles and straw. The nematode remains viable in this state in dry tissues and under hulls of rice grains for up to 3 years (Todd and Atkins 1958; Fortuner and Orton-Williams 1975). At tillering stage, the number of nematode increases rapidly and reaches a peak during the reproductive stage of the plant. Damage to the outer wall of the ovary causes partial filling of kernels and damage to the lodicules which prevent closure of flower after anthesis, exposing the embryo to environmental stresses like desiccation (Rao and Rao 1979).

Survival of *A. besseyi* is inversely related to the extent and rate of dehydration and the nematodes in larger aggregates were found to survive better than those in the smaller ones. The starvation adversely affects the ability of the nematode to survive dehydration. Larvae and adults of the nematode were found equally capable of withstanding desiccation (Huang and Huang 1974). Detection of *A. besseyi* entails the soaking of seed in water for 12–24 h and examination of the suspension for reactivated nematodes.

8.2.3 *Ditylenchus*

Another important genus is *Ditylenchus*, having two important species *Ditylenchus dipsaci* and *Ditylenchus destructor.* Both the species affect certain plants (e.g. beets, lucerne, clover), but in general these two species rarely occur together in the same plant (Andrássy and Farkas 1988). The other important species is *Ditylenchus angustus,* known as foliar nematode of rice, which can be transmitted in dried rice foliage.

8.2.3.1 *Ditylenchus dipsaci*

Ditylenchus dipsaci, or stem nematode, attacks more than 1200 species of wild and cultivated plants. Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. *D. dipsaci* lives mostly as an endoparasite in aerial parts of plants (stems, leaves, and flowers) but also attacks bulbs, tubers, and rhizomes. This nematode is seed-borne (Sousa et al. 2003; Sikora et al. 2005) and survives in infected seed (Palmisano et al. 1971). The great importance of this nematode is the fact that the fourth stage juvenile can withstand desiccation for a long time, sometimes 20 years or more (Barker and Lucas 1984). The presence of the infective fourth stage juveniles in seed and dry plant material is important in the passive dissemination of the nematode over long distances. These nematodes clump together in a cryptobiotic state to form "nematode wool" when the plant tissue begins to dry. The wool can often be observed on the seeds in heavily infested pods and in dry plant debris.

8.2.3.2 *Ditylenchus destructor*

Ditylenchus destructor, or potato rot nematode, attacks the subterranean parts of plants (e.g. tubers, rhizomes, and stemlike underground parts). The host range of the nematode is extensive, comprising more than 90 plant species, which include ornamental plants, crop plants, and weeds. *Solanum tuberosum* (potato) is the principal host, the tubers develop wet or dry rot that further spreads to other tubers in storage causing discolouration and rotting of the tissues. The above-ground parts are sometimes also infected, causing dwarfing, thickening, and branching of the stem and dwarfing, curling, and discolouration of the leaves (Sturhan and Brzeski 1991).

The nematodes enter potato tubers usually via the stolons. Most of the nematodes are located at the edge of the browning and undamaged parts. If a small sample from this part of the tuber is taken and placed in water, the mass of small nematodes is conspicuous even with a simple magnifying glass. The earliest symptoms of *D. destructor* infection are small, white, chalky, or light-coloured spots that can be seen just below the skin of the tuber. These spots later become larger and gradually darker (through grey, dark brown, and black) and acquire a spongy texture. On severely affected tubers, there are typically slightly sunken areas with cracked, wrinkled, papery skin. The skin becomes thin and cracks due to dryness

and shrunken infected tissues (Brodie 1998). Finally, mummification of whole tubers may occur and such fully damaged tubers float in water. Symptoms may be more visible after storage when the nematodes continue to reproduce inside the tubers after harvest and may build up to large numbers.

Ditylenchus destructor is an important pest of groundnut (Jones and De Waele 1988; De Waele et al. 1989). The groundnut hulls show black discolouration along the longitudinal veins. The infected seeds are shrunken, while the testa and embryo of seed show yellow to brown or black discolouration. The developing pods are invaded by nematode just after the fruiting pegs have penetrated the soil. The nematodes then enter the exocarp, feed on the parenchyma cells, and migrate to the base of the mesocarp. They remain confined there and discolour the adjoining cells. After the breakup of mesocarp, nematodes reach the endocarp and enter the seed through the micropyle, then invade the seed coat and embryo, and are observed on the surface of the cotyledons.

8.2.3.3 *Ditylenchus angustus*

Ditylenchs angustus causes 'ufra' disease in rice, and the most prominent symptoms of infected plants are chlorosis, twisted leaves, and swollen lower nodes. Infected panicles are usually crinkled with empty, shrivelled glumes, especially at their bases, and the panicle head with flag leaf is twisted and distorted (Bridge et al. 2005). *Ditylenchus angustus* survive in a dried state enclosed in the leaf sheath, particularly at the base of the panicle. In the natural state, *D. angustus* can survive for 4–5 month in dry foliage (Cox and Rahman 1979; Dang-ngoc 1981) or found inside the filled and unfilled grains of freshly harvested rice, but the nematodes are apparently killed when the seed is sun-dried (Sein 1977; Nguyen-thi and Giang 1982). However, these nematodes may also spread by infested field soil.

8.2.4 *Pratylenchus brachyurus*

The lesion nematode, *Pratylenchus brachyurus*, is a widespread and dangerous parasitic nematode throughout the tropics and subtropics, causing major crop losses in pineapple, soybean, groundnut, potato, and maize (Godfrey 1929; Ferraz 1999; Silva and Pereira 2003). Lesions were observed in the roots, which were identical to the irregularly elongated brown spots observed by Godfrey (1929). The *Pratylenchus* species causes necrosis of the roots, attacking primarily the fibrous root, thus reducing the absorption of water and nutrients by the plant. Infected plants exhibit stunting and poor vigour. Poor root development is reflected in poor development of the aerial parts of the plant, causing severe damage and resulting in reduced crop yield. In the field, visual symptoms are manifested in the form of 'patches' or 'foci' of stunted plants with chlorosis and varying degrees of nutritional deficiency, as well as dry leaf edges, in contrast with surrounding plants that do not have visual symptoms of nutrient deficiency (Cadet and Spaull 2005).

8.2.5 *Bursaphelenchus* (*Rhadinaphelenchus*) *cocophilus*

Red ring nematode *Bursaphelenchus cocophilus* formerly known as *Rhadinaphelenchus cocophilus* causes red ring disease in coconut and palm. *Bursaphelenchus cocophilus* parasitizes the palm weevils which transmit it to coconut palm and oil palm. They are carried by seeds and seedlings and can survive in nut husk up to 16 weeks and 19 weeks in infected seedlings tissues. Internal damage can be seen within 2–3 weeks after *Bursaphelenchus cocophilus* enters the tissue of a healthy palm. The major internal symptom of infection is the "red ring" for which the disease was named.

In external symptoms, already established leaves become short and deformed and turn yellow bronze, then deep reddish brown. The colour change usually begins at the tip of each leaf and starts in the older leaves before moving to the younger ones. As the leaves change colour and dry up, they wilt and die. New leaves often become shorter as the disease progresses, causing the central crown of the tree to resemble like a funnel. Eventually, these new "little leaves" display varying degrees of necrosis and stop producing fruit.

In coconut palms, red ring nematodes most often attack trees between 3 and 7 years age. These young trees usually die 6–8 weeks after the appearance of symptoms. Older trees can survive up to 20 weeks (Esser and Meredith 1987). Red ring nematodes invade both palm tissue and roots. In leaves, stem, and roots, they block water pathways, reducing the palm's water absorption. The heaviest concentration of nematodes can be found at the internal red ring that is a classic symptom of red ring disease.

8.3 Detection and Diagnosis Methods of Seed-Borne and Seed-Associated Nematodes

Detection and identification of nematodes are the first step in controlling them and restricting their spread. Detection and management of seed-borne diseases through quality control programmes that monitor seeds from harvest to purchase, marketing, and sowing in the field are essential to ensure high quality and genetically pure seed.

Detection of the nematodes is based primarily on conventional methods, viz. direct inspection of dry seeds, washing test, soaking test, incubation tests, blotter tests, embryo count test and filter, and centrifuge extraction technique. These methods had been in regular practice till last decades of twentieth century when molecular detection and diagnosis approaches have taken lead, even though conventional methods have its own importance being simple and non-expensive.

Nucleic acid-based molecular approaches (Chahal and Pannu 1997; Lopez et al. 2003), which are specific, rapid, and reliable, were developed for accurate and rapid detection of seed-borne pathogens. Molecular diagnostics began to develop a real momentum after the introduction of PCR in the mid-1980s. Diagnostic assays based

on PCR have been developed for major seed-borne nematodes. Development of genomic techniques for characterization of plant pathogens during the past decades has greatly simplified and improved pathogen detection and identification.

8.3.1 Conventional Methods

The conventional methods are mostly based on interpretation of visual symptoms, culturing, and laboratory identification. These methods require extensive taxonomic expertise, are time-consuming, and are difficult to diagnose samples with hidden symptom. Even then, some classical simple diagnostic techniques have been applied to seed samples for a long time which are preliminary and less expensive for detection and still have much importance.

8.3.1.1 Visual Inspection of Seeds Infected by Nematode

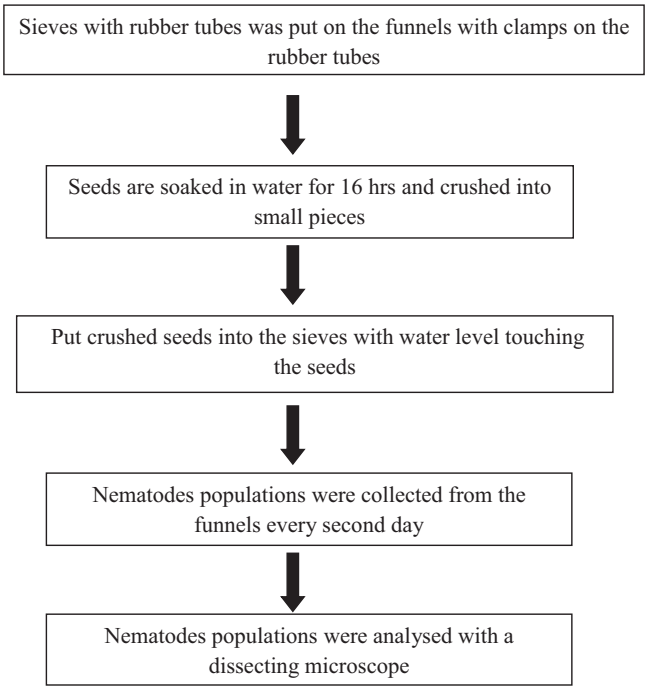
(a) *Dry Seeds*

There are several visual inspection procedures which can be used successfully to detect a number of plants infected by nematodes. There is no instrument as sophisticated as human eye. Our eye can detect the stable variation in colour, dimension, etc., which could give a clue for a possible presence of nematodes in seed material. Morphological abnormalities on seeds or plants can be easily detected by examining seed samples in daylight for particular effects of nematodes on discolouration, pigmentation, etc. which can be detected by naked eye or by the use of optical lenses (De Tempe and Binnerts 1979). This procedure provides quick information and may be applied along with routine testing, provided that it is practiced well. This procedure is applied to detect immature stages as well as adult nematode infection manifested as galls and cyst of nematodes. The roots for galls, lesions, cyst, abnormal development of secondary root, poor growth, or hairy growth can also be examined by use of magnifying glasses. Presence of galls and lesions indicates nematodes of *Meloidogyne* and cyst-forming nematodes such as species of *Globodera* and *Heterodera,* respectively. For example, yellow ear rot or tundu disease-infected wheat seeds can be detected by visual inspection of black galls which are due to nematode *Anguina tritici* (Steinbuch) Chitwood. Appearance of galls or cockles caused by nematode on wheat seeds are small, dark purplish, black, or dull-coloured. The diagnosis is confirmed by soaking galls in water for 1 h and cutting them into pieces in drops of water. Active larvae are released into the water and can be seen under a binocular microscope. Cyst of nematodes can be observed and detected by dipping rhizospheric soil or plants in water, where it floats on the surface of water and can be seen with the help of magnifying glasses. The cyst can be collected by a brush and mounted on the microscopic slide in a drop of water. These cysts, both alive and dead, can be determined by breaking them and examining under a microscope.

(b) *Seed Soak Method*

This is a simple method which can be used to detect both endoparasitic and ectoparasitic nematode, dead or alive. This method is useful in isolating and detecting nematodes on seeds and other plant tissues. Seeds or tissues and plant parts are cut into small pieces, soaked in water, and kept for 24 h to be examined under a stereo binocular microscope. This is the simplest method to detect stem and bulb nematodes *Ditylechus dipsaci* and leaf gall nematodes *Aphelenchoides besseyi* which forms a white encrustation on leaves. This method has limitation in that the nematodes collected are only of juvenile form, and in plant tissues or seeds, the distribution of nematodes is uneven and small in number, so sometimes it is not useful for correct diagnosis.

Flow chart for the extraction of seed borne nematode



(c) *Floatation Method*

This method is useful in detecting nematodes from cyst, galls, and similar structures which float in water. This is a quick method to detect cyst/galls of nematodes or egg masses of nematodes. The material to be examined is placed in the salt water of 8% NaCl and stirred continuously for 10 or 15 min before keeping

it for 24 h without disturbing it. After this period, the solution is allowed to pass through the funnel containing filter paper for complete drain; then the sediment on the paper is examined for nematode cyst, galls, or egg masses of nematodes. Seeds infected with tundu disease can be detected by this method. Thousands of juvenile nematodes (*A. tritici*) can burst from the gall upon piercing with a needle. This method is useful only where structures are in free state and able to float on the surface.

(d) *Washing test*

Some nematodes associated with the planting material adhere in aggregated or scattered form in the soil particles in root zones. Unless they are removed, collected, and concentrated from the plant tissues, it is not possible to diagnose them properly. The simple method to detect them is to wash them and collect them in water. By examining a drop of water obtained from washing of soil sample and collecting them after sieving, the nematodes can be detected under compound microscope which gives an indication of nematode population associated with seed/planting material.

8.3.1.2 Maceration Method for Nematode Extraction

(a) *Maceration*

In case of woody plant tissues, it is difficult to extract nematodes, which are deep-seated. Unless the tissues are examined, it may not be possible to detect the nematodes. For such a condition, maceration method is useful in detecting nematodes which are deep-seated in root tissues, bulb, tubers, corms and woody plant, etc. Small part of infected tissues is macerated in 100 ml water in a blender for 10–50 s. After maceration, the macerated tissues are shifted for nematode collection to the modified Bayernann funnel and examined for nematodes' presence (Coolen and D'Herde 1972).

For the nematodes which generally inhabit xylem and other tissues surrounding the xylem, for example, red ring nematodes *Rhadinaphaenchus cocophilus* in palm, can be detected only by collecting some infected tissues by chopping them well and then macerated in 100 ml of water for 15 s in a blender. The suspension is then kept in flask for 30 min; the heavier particles will be settled down, and the nematodes and few light weight particles will float which are sieved 3–4 times into 250 mesh sieves (Seinhorst 1988).

(b) *Root Incubation Method*

This method is generally useful for migratory endoparasites. There are some nematodes, which remain inside the plant tissues and cannot be separated even if the tissues are macerated. In order to diagnose these nematodes, the plant tissues are to be incubated for certain period. This method is generally useful to detect migratory endoparasite such as *Radopholus* and *Pratylenchus*. Roots of banana, citrus, coffee,

chrysanthemum, etc. may be examined for *Radopholus* and *Pratylenchus*. The roots are washed thoroughly and cut into small pieces by splitting longitudinally. The roots are wetted by submerging them in a thin layer of water and then the roots are kept in a closed container such as petri dishes and incubated at 20–25 °C temperatures for 24 h. The incubated Petri dishes are then observed for nematodes under stereo binocular microscope for diagnosis (McSorley et al. 1984).

(c) *Centrifugation*

Centrifugation technique is used to detect nematodes in suspension where lighter particles fly off from the centre. This tendency or centrifugal force is being extensively used in separation of nematodes associated with soil particles or tissues and separated on the basis of sedimentation at 2000 rpm. This technique can be used only to aggregate the scattered nematode population and visualize them under microscope.

8.3.2 Serological Techniques

8.3.2.1 General Concepts of Immunoassay

The term serology is used to refer to the diagnostic identification of antibody in blood serum. But in plant pathology, serology is used for the detection and identification of any antigenic substances or pathogenic entities. The serological techniques are mainly based on antigen-antibody specificity, i.e. binding ability of an antibody to its corresponding antigen molecules. Like other phytopathogens, monoclonal antibodies (MAbs) and polyclonal antibodies (PAbs) are generated for many plant-parasitic nematodes, and they are used for serodiagnostic purpose. These antibodies are developed against diverse antigenic determinants present in nematodes' surface-coat and excretory/secretory (ES) products. In many nematode species, the surface coat (SC) is composed mainly of proteins, carbohydrates, and lipids (Spiegel and McClure 1995). The surface-coat proteins of the plant-parasitic nematodes mostly include carbohydrate-binding proteins and glycol proteins, which act as the antigenic determinant or epitope (Dell et al. 1999). Characterization of epitope is essential for antibody development. These glycosylated epitopes are predominant on the parasite surface and need to be tested for their antigenic specificity and cross-reactivity. Here, antigenic specificity determines the ability of immune system to differentiate between different antigens. The antigen may have some unique sequences or signature domains which are specifically used for diagnostic and the cross-reactivity measures, the extent to which same epitope appears similar to different antigens. The molecular determinants of antigenic specificity and cross-reactivity are determined by the nature of antigenic variation.

Here, polyclonal antibodies show higher level of sensitivity but are not usually discriminative due to cross-reactivity across nematode genera. Therefore, polyclonal antibodies are unable to distinguish different race/strains of the same phytopathogenic nematode. Production of a monospecific polyclonal antibody to a diagnostic protein

can overcome some of the problems with cross-reactivity. But production of sufficient amount of pure antigen is always challenging. Moreover, antisera produced in different batches may react differently in the same assay. Therefore, in spite of low production cost for polyclonal antibody, monoclonal antibodies are preferred for immunodiagnostic assay due to better strain/race discriminating ability. Monoclonal antibodies (MAbs) produced from cell lines can give high specificity and better reproducibility between batches, but their production is expensive and cell lines can be unstable. Screening existing libraries for an antibody that has the required specificity is another alternative but requires technical expertise.

8.3.2.2 Antibody Production Techniques

For the identification of plant-parasitic nematode, polyclonal and monoclonal antibodies have been produced against antigen derived from respective nematode. Mostly, homogenates of cysts or juveniles, purified proteins originated from surface coat, and secretions of nematodes are used for antibody development. The sample population of nematode from which the antigen will be extracted, it's life-stage is important for antibody sensitivity and specificity. After extraction, antigen homogenate prepared in phosphate buffer and then may be mixed in detergent (0.1% Triton X-100). Both crude homogenates and purified protein extracts are used for immunization into rabbit or mice. Preparation of crude antigen homogenates is quite easy. However, use of purified nematode proteins will probably be essential in order to improve the specificity of antibody response. Protein purification is done by SDS-PAGE. Antibody production is found to be better when particulate form of antigen is used than soluble form. Sometimes, use of detergent may reduce the chance of low cross-reactivity of produced antibody. Before immunization, antigen has to be emulsified with equal volume of complete Freund's adjuvant and then administrated intraperitoneally in mice or intramuscularly in rabbit, with two subsequent booster injections with incomplete Freund's adjuvant (Davis et al. 1992). The adjuvant acts as a carrier of antigen and ensures uniform distribution to various antibody production sites. Use of adjuvant promotes high titre of antibody response. More than one time immunization is always preferred for getting better response. When highest titre of antisera will reach, animal would be euthanized and antisera will be collected. In this way, novel methods for monoclonal and polyclonal antibodies development have also been formulated against large number of plant-parasitic nematode.

8.3.2.3 Immunodiagnostic Techniques

A vast array of immunodiagnostic techniques is available and has been tested for the detection and diagnosis of large number of plant pathogens (Table 8.2). They mostly include indirect immunofluorescence assay, passive haem-agglutination assay, enzyme-linked immunosorbent assay (ELISA), etc. For routine testing such as for screening of germplasm and planting material, enzyme-linked immunosorbent assay (ELISA) is the most appropriate tool. In comparison with other serological methods, it is very easy, relatively inexpensive, semi-quantitative in nature, and can be automated for large-scale testing. Sometimes, it has an edge over molecular

Table 8.2 Immunodiagnostic techniques employed for the detection of seed/planting material-borne phytonematodes

Immunodiagnostic technique	Antibody used	Nematode species	Detection stage	References
ELISA	MAbs	*Heterodera glycines*	Juveniles	Atkinson et al. (1988)
ELISA	MAbs	*Ditylenchus dipsaci*	Juveniles & Adult	Palmer et al. (1992)
ELISA	MAbs	*Globodera rostochiensis*	Juveniles	de Boer et al. (1996)
DAS-ELISA	MAbs	*Heterodera avenae*	Juveniles	Curtis et al. (1997)
Indirect ELISA	MAbs, PAbs	*Heterodera glycines*	Egg mass	Kennedy et al. (1997)
Competitive inhibition ELISA	PAbs	*Meloidogyne incognita*	Egg mass	Kapur-Ghai et al. (2014)
Indirect ELISA	Anti-*M.incognita* Antibodies	*Meloidogyne incognita*	Egg mass	Kapur-Ghai et al. (2014)

diagnostic methods, as it is faster and any skilled person can perform this. So far, ELISA has been employed by many workers for detection of various plant pathogenic nematodes, mostly based on specific monoclonal antibodies. ELISA, based on monoclonal antibodies, is inexpensive and very sensitive as well as specific. Thus, it can easily distinguish different nematode species infecting same specimen and it is feasible to detect infection of nematode as low as one larva/100 g of tissue. However, when dealing with unknown nematode specimen or any invasive alien species, such as in surveys or in quarantine station, immunoassays are usually not the most appropriate technique to use.

8.3.2.4 Protein-Based Techniques

Protein profiling was used as an alternative approach to serodiagnostic of phytonematodes. This involves identification of novel proteins with specific origin. It needs separation of nematode proteins via two-dimensional gel electrophoresis (2DE) to generate diagnostic protein profiles, followed by identification of certain unique proteins using Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS), which provide signature protein spectra/sequence for species/race identification. Use of MALDI-TOF MS is very novel for generating protein profiles that would be useful for diagnostic of plant nematodes. Diagnostic species-specific protein peaks present in the spectra are identified for each species. This technique enables inter- and intra-specific differentiation of plant-parasitic nematode and also helps to identify alien phytopathogenic nematode species for biosecurity concern. Such protein profiles were generated for *Anguina tritici, A. funesta, Globodera rostochiensis, Heterodera glycines*, *H. schachtii*, *H. avenae, Meloidogyne javanica,* root lesion nematodes (*Pratylenchus* spp.), etc. (Perera et al. 2005; Tan 2012). A disadvantage of protein-based techniques is that cellular expression of proteins is influenced by environmental factors and often relies on

developmental stage of the organism. Moreover, it may not be uniform in all individuals within nematode population. Since DNA-based diagnostic methods do not rely on the expressed products of the genome, and are independent of environmental influence, independent of stage in the nematode's life cycle, and potentially extremely discriminating, they provide attractive solutions to the problems associated with protein-based identification methods (Tan 2012).

8.3.3 Molecular Techniques

Molecular biology has revolutionized the field of systematics, genomics, and diagnostic of phytopathogenic nematodes. Different molecular techniques have been used for the identification of plant-parasitic nematodes and they provide accurate, reliable diagnostic information. Initially, these techniques were solely applied for deciphering taxonomic identity of certain nematode species, but increasingly became popular for rapid and robust diagnosis of infected plant samples. Now-a-days, techniques like PCR-RFLP, PCR with specific primers, real-time PCR, LAMP, and DNA sequencing are most widely used for diagnostic purpose. Besides these, techniques like 'DIRT(s)'are available to evaluate nematode population of individual species by extraction of DNA directly from soil. Different molecular approaches resolving nematode diagnostics are presented and discussed, here.

8.3.3.1 Hybridization-Based Techniques

Basically, RFLPs technique involves restriction digestion of genomic DNA, followed by visualization of banding patterns in gel electrophoresis. This polymorphism in fingerprinting is used to distinguish species and isolates/race/strain of plant-parasitic nematodes. Initially, this RFLP technique is applied for detection of *Meloidogyne* spp. (Curran et al. 1985). This technique had greater discriminatory potential than serological and biochemical approaches (Nega 2014). Later, RFLPs were combined with hybridization technique using radioactive or a non-radioactive-labelled DNA probes for identification of *Ditylenchus dipsaci* (Palmer et al. 1992) and for species differentiation in potato cyst nematode (*Globodera pallida, G. rostochiensis*) (Marshall and Crawford 1987; Burrows and Perry 1988). However, lack of sensitivity in interspecific discrimination by this technique restricted its applications. The introduction and development of polymerase chain reaction (PCR) has replaced the hybridization-based approaches for nematode identification.

8.3.3.2 PCR-Based Technique

(a) *Conventional PCR Using Molecular Marker*

Introduction of PCR has contributed to the rapid increase of identification techniques that are relatively fast and require minute quantities of DNA and, very often, can be applied on individual nematodes. Considerable effort has been made in

designing PCR-based identification methods for plant-parasitic nematodes. Such methods are based on either targeted amplification of genomic and mitochondrial DNA, or random amplification of polymorphic DNA fragments.

The amplification of specific genomic regions is a highly effective way of detecting inter- and intra-specific variations between genera and species and within species (Abrantes et al. 2004). The ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) are most commonly targeted for taxonomic and diagnostic purposes. Ribosomal DNA (rDNA) is the most important genomic segment for comparative studies of evolutionary and phylogenetic aspects (Vrain et al. 1992; Ferris et al. 1993). It contains different conserve and variable domains with similar basic organization across the eukaryotes. Eukaryotic ribosomal RNA genes appear in tandem repeat forming cluster. The basic unit consists of three genes for the small ribosomal subunit, the 5.8S subunit, and the large ribosomal subunits separated by two internally transcribed spacers (ITS-1 & ITS-2) (White et al. 1990). However, each basic unit within gene cluster is separated by an intergenic spacer (IGS) (Hillis and Dixon 1991). Highly conserved regions in the ribosomal repeat array including large subunit (LSU) and small subunit (SSU) genes can be used to study relationships across phyla (Gerbi 1985), whereas variable regions, i.e. internal transcribed spacers (ITS) and intergenic spacers (IGS), are used at lower taxonomic rank. The ITS region does not encode for any product, but evolve at a faster rate than the ribosomal coding regions, thus representing as considerably more variable regions. The higher level of variation in this region makes it suitable for molecular systematics and diagnostic study at the species level within a single genus and even within species. The genomic variation in the rDNA ITS region can be detected either based on size variation or sequence variation. Size variation can only be visualized using gel electrophoresis, but may not be sufficient for identifying plant-parasitic nematodes. To avoid such limitation, PCR amplification of internal transcribed spacer regions (ITS 1 and ITS 2) combined with restriction digestion using restriction endonuclease (PCR-RFLP) yields patterns in gel, which is a highly discriminating tool and used for nematode diagnostics. It has been used to characterize several species of *Tylenchida*, *Longidorus, Paralongidorus, Xiphidorus,* and *Xiphinema* (Table 8.3).

Other techniques involve random amplification of DNA fragments using a set of primers that will generate polymorphism. This polymorphism in DNA sequences is unique for a particular taxon and appears due to genomic variation. It is considered as molecular marker. Based on their functioning, they are basically categorized as random markers and locus-specific markers. Random molecular markers do not require any genomic information and locus of the amplicons is absolutely unknown. This category of markers includes random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), etc. These markers help to screen the whole genome profiling-based variation. These can be used to detect random polymorphism within genome for identifying species-specific sequences, when variation within conserved genes is not enough to successfully discriminate species. For example, RAPD marker has been used for interspecific and especially intra-specific discrimination, particularly of *Globodera* and *Meloidogyne* species (Abrantes et al. 2004). Using RAPD-PCR

Table 8.3 Molecular diagnostic technique used for the identification of plant-parasitic nematode

Diagnostic technique		Target genes/region	Nematode species	Purpose	References
Hybridization based	RFLP	Genomic DNA	*Ditylenchus dipsaci*	Pathogen identification	Palmer et al. (1992)
	RFLP	Genomic DNA	*Globodera pallida*, *G. Rostochiensis* (potato cyst nematode)	Species discrimination	Marshall and Crawford (1987) and Burrows and Perry (1988)
	RFLP	Genomic DNA	*Heterodera glycines* (soybean cyst nematode)	Race profiling	Kalinski and Huettel (1988)
	RFLP	Alcohol dehydrogenase gene	*Bursaphelenchus xylophilus* (pinewood nematode)	Discrimination of pathogenic and non-pathogenic species	Bolla et al. (1988)
PCR based	PCR-RFLP	Ribosomal DNA (rDNA)	*Aphelenchoides* spp.	Species differentiation	Ibrahim et al. (1994)
		Ribosomal DNA (rDNA)	*Ditylenchus angustus* (Ufra disease of rice)	Pathogen identification from mixed infection in rice	Ibrahim et al. (1994)
		ITS1 of ribosomal DNA (rDNA)	*Anguina* spp. (seed gall nematodes)	Identification of *anguinid* species for regulatory concern	Powers et al. (2001)
		Ribosomal DNA	*Bursaphelenchus xylophilus, B. mucronatus* (pinewood nematodes)	Differentiation of two similar nematode species	Iwahori et al. (2000)

(continued)

Table 8.3 (continued)

Diagnostic technique		Target genes/region	Nematode species	Purpose	References
	Conventional PCR	RAPD	*Globodera* spp., *Meloidogyne* spp.	Interspecific and especially intra-specific discrimination	Abrantes et al. (2004)
		SCAR	*M. arenaria, M. incognita, M. javanica*	Identification of different species	Zijlstra (2000a, b) and Zijlstra et al. (2000)
		SCAR	*Meloidogyne chitwoodi, M. fallax*	Identification of nematode in population	Zijlstra (2000a, b)
		Species-specific primers	*Aphelenchoides fragariae*	Identification of *A. fragariae* directly plant tissues and discrimination from other *Aphelenchoides*	McCuiston et al. (2007)
	Multiplex PCR	Specific primer sets (Pc1-Pc2)	*Pratylenchus coffeae*	Species identification	Uehara et al. (1998)
		Specific primer sets (Mi1-Mi2 & Pc1-Pc2)	*Meloidogyne incognita, Pratylenchus coffeae*	Identification of different nematode species	Saeki et al. (2003)
		Species-specific SCAR markers	*Ditylenchus dipsaci*	Identification of the giant and the normal types of *D. dipsaci*	Esquibet et al. (2003)
		Species-specific SCAR markers	*Meloidogyne ethiopica*	Distinguish *M. ethiopica* from other root-knot nematodes of grapevine and kiwifruit seedlings	Correa et al. (2014)

	Real-time PCR	Heat shock protein (hsp90) gene	*Ditylenchus dipsaci, D. gigas, D. weischeri* (stem nematodes)	Simultaneous detection and differentiation of *Ditylenchus* spp.	Sun et al. (2016)
		Species-specific primer based on ribosomal DNA	*Anguina funesta, A. agrostis, A. tritici,* and *A. pacificae*	Identification of different *Anguina* spp.	Li et al. (2015)
		ITS2 of ribosomal DNA	*M. chitwoodi* and *M. fallax*	Simultaneous detection in potato tuber	Zijlstra and van Hoof (2006) and de Haan et al. (2014)
		Heat shock protein (hsp70) gene	*Bursaphelenchus xylophilus* (pine wood nematode)	Detection and differentiation from other wood-inhabiting nematode species	Leal et al. (2007)
	LAMP-PCR	rRNA gene D2-D3 regions	*Radopholus similis* (burrowing nematode)	For early diagnosis of infested plant tissues	Peng et al. (2012)
		ITS sequences	*Meloidogyne hapla*	In situ diagnosis of infected plant	Peng et al. (2017)
		5S rDNA-IGS2 regions	*Meloidogyne enterolobii*	Practical tool quarantine diagnosis	Niu et al. (2012)

(continued)

Table 8.3 (continued)

Diagnostic technique		Target genes/region	Nematode species	Purpose	References
Sequencing based	Barcoding	IGS	*Meloidogyne chitwoodi, M. fallax*	Distinguish root-knot nematode species	Petersen et al. (1997)
		ITS	*Heterodera schachtii*	Identification of nematode species	Amiri et al. (2002)
		Satellite DNA	*Bursaphelenchus xylophilus* (pinewood nematode)	Species-specific probe	Castagnone et al. (2005)
		Cytochrome c oxidase subunit 1 (COI)	Non-americanum-group of *Xiphinema* spp. as well as *Paralongidorus* spp. and *Longidorus* spp.	Species discrimination and universal barcode	Palomares-Rius et al. (2017)
		Cytochrome c oxidase subunit 1 (COI)	*Bursaphelenchus* spp.	Species discrimination	Ye et al. (2007)
		Nad5 (mitochondrial coding genes)	*M. incognita, M. javanica, M. arenaria* (tropical root-knot nematodes)	Species identification	Janssen et al. (2016)
	Whole genome sequencing	–	*Meloidogyne incognita, M. hapla*	–	Abad et al. (2008) and Opperman et al. (2008)
		–	*Globodera rostochiensis*	–	Eves-van den Akker et al. (2016)
		–	*Bursaphelenchus xylophilus*	–	Kikuchi et al. (2011)
Array based	Microarray	Oligo-nucleotide probe	*Meloidogyne chitwoodi*	Detection of *M. chitwoodi* from mixed infection of *Meloidogyne* spp.	François et al. (2006)
	Microarray	Padlock probe	*Meloidogyne hapla*	Sensitive and multiplex detection of 10 economically important plant pathogens	Szemes et al. (2005)
	Open arrays	Padlock probe	*M. hapla*	Quantitative and simultaneous detection of 13 plant pathogens	van Doorn et al. (2007)

approach, some of the amplified DNA fragments that were found to be specific for the major species (i.e. *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*) were further cloned and transformed into SCAR markers to provide reliable tools for the identification of these economically important pests (Zijlstra 2000a, b; Zijlstra et al. 2000). However, gene specific markers are used to amplify certain specific genomic regions using a set of primers for the detection of polymorphism. These molecular markers need prior genomic information and can be derived from genomic DNA libraries, SSR library, EST library, whole genome sequence, and transcriptome database. It mostly includes Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs), etc.

(b) *Multiplex PCR*

Though PCR has revolutionized the field of plant disease diagnosis, it has certain disadvantages like high cost and time requirement for each sample. To improve the diagnostic capacity of the PCR-based tests, multiplex PCR, a variant of PCR, was introduced in which simultaneous amplification of more than one target sequence is possible using specific sets of primer combinations (Elnifro et al. 2000). Multiplexed PCR involves primer mixtures consisting of specific set primer pairs for each target, or single primer pairs that allow differential amplification of several targets. Thus, it facilitates detection of number of pests and pathogens at a time with reduced cost and time. Designing of species-specific primers needs the broad knowledge of sequence divergence within population of the same species and in closely related species. Although it may considerably save time and effort without compromising test utility, it requires precise optimization of the reaction conditions for the primer sets used for simultaneous amplification. This technique is most commonly used for identification and discrimination of different species of *Meloidogyne* (Hu et al. 2011), *Pratylenchus* (Uehara et al. 1998), *Xiphinema* (Wang et al. 2003), and *Ditylenchus* (Esquibet et al. 2003).

(c) *Real-Time PCR*

During the early 1990s, a new variant of PCR was introduced for quantification of amplicons in real time. It is used for identification purpose and has advantages of increased sensitivity (compared to conventional PCR), simultaneous detection of more than one species, and the absence of post-PCR processing steps. Now-a-days, it is most commonly used for detection and species discrimination of *Meloidogyne* (*M. chitwoodi* and *M. fallax*) which will help in quarantine purpose (Zijlstra and van Hoof 2006).

8.3.3.3 Sequencing-Based Technique: DNA Barcode

The DNA barcode represents a signature nucleotide sequence of common gene(s) that can serve as a unique identification code for every individual species. It is a very promising approach for identification of plant pathogenic nematode. DNA barcode includes not only one but sometimes two, or even more genes/genomic regions,

which must show significant degree of genetic variability at species level. Basically, a short sequence is preferred that must have conserved flanking sites for developing universal primers and easy amplification. Several DNA regions, such as 18S rRNA gene (Floyd et al. 2002), D2–D3 expansion segments of 28S rRNA (De Ley et al. 2005; Subbotin and Moens 2006), or the ITS of rRNA gene (De Ley et al. 2005; Subbotin et al. 2005), have been proposed to generate DNA barcode. Among these gene, ITS is mostly preferred for reliable diagnostic of large number of plant-parasitic nematode genera, viz. *Heterodera, Globodera, Bursaphelenchus, Pratylenchus, Anguina, Ditylenchus, Nacobbus,* and *Radopholus* (Kaur 2016). In addition to ITS, inter-genic spacer (IGS) of nuclear rRNA between 28S and 18S rRNA genes (Petersen and Vrain 1996) is used for species identification of root-knot nematodes. Other than nuclear DNA, regions of mitochondrial DNA which include Cytochrome C Oxidase subunit 1 (COI), the intergenic region between the Cytochrome Oxidase II (COII), and the large sub-unit of the RNA gene (lrRNA) provide accurate information for species identification across the kingdom (Hebert et al. 2003) and are already tested for *Meloidogyne* species diagnostics (Powers and Harris 1993). Some other genes like heat shock Hsp90 (Skantar and Carta 2004) and actin (Kovaleva et al. 2005) are also used for diagnostic purposes.

8.4 Application of Detection Techniques for the Management of Seed-Borne Nematodes

8.4.1 Strengthening Seed Certification Programme

Detection of nematode in seeds and other propagative materials is very crucial for the management of phytonematodes. The detection of seed-borne nematode using conventional methods is not only time-consuming but less accurate. Therefore, detection along with quantification of level of seed infection/contamination is very essential for seed certification programme. ELISA can be used for rapid, sensitive, and specific detection of nematodes associated with seed but provides semi-quantitative information. Real-time PCR (qRT-PCR) is an excellent diagnostic tool for specific and rapid quantification of plant-parasitic nematodes. It can be applied successfully in soybeans for accurate diagnosis of soybean cyst nematode (*Heterodera glycines*) in a short period of time. It is also used for species discrimination of *Anguina agrostis*, *A. funesta*, *A. tritici*, and *A. pacificae* (Li et al. 2015). Hence, this technology should be more widely employed for routine diagnostics in seed certification programme.

8.4.2 In Situ Diagnosis and Management of Phytonematodes

Production of nematode-free seeds is always associated with in situ diagnosis of plant and production of clean crop. For the management of seed-borne nematode, regular survey/monitoring over a defined period of time should be done for the

selection of nematode-free crop and establishment of pest-free area. Harvest of the certified area should be sampled for fixed number during cropping season. Fixed number of samples should be randomly taken for detection. Strong inspection programme, coupled with frequent sampling, testing, and verification of information, is a prerequisite for appropriate phytosanitary actions.

8.4.3 Quarantine Detection and Protection from Invasive Nematodes

In the era of WTO, free international trade and unrestricted movement of seed and planting materials may accelerate the introduction of new pests and pathogens in an un-invaded area and may cause devastation of crops as evident in past. To protect agriculture, this trans-boundary movement should ensure safe import of seeds and exchange of germplasm. Therefore, quarantine detection of invasive alien nematodes should be strengthened for exporting raw planting materials, and exporting agencies should meet certain phytosanitary standard as prescribed by importing countries. On the other hand, for thorough check-up of imported bulk consignments, suitable diagnostic techniques should be included in a quarantine programme. These techniques should be relatively simple and robust, with higher resolution of detection. Moreover, amalgamation of conventional technique with advanced molecular tools like ELISA or PCR increases diagnostic accuracy, which is essential to minimize the risk of entering internationally important quarantine nematode pests like *Bursaphelenchus xylophilus* (pine wood nematode), *Ditylenchus dipsaci* (stem and bulb nematode), *Aphelenchoides arachidis* (groundnut testa nematode), *Anguina agrostis* (bent grass nematode), and *Rhadinaphelenchus cocophilus* (red ring nematode). In this genomic era, development of more sophisticated diagnostic tools like universal DNA chip has become urgent for large-scale application and detection of quarantine pests.

8.5 Conclusion

Seed is a small embryonic plant which is a basic unit of production for most of the world's food crops. It is an efficient means of introducing pathogens into a new area as well as providing support for their survival from one cropping season to another. Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest. Seed-borne nematodes are one of the most important biotic constraints in many of the crops/seed production systems worldwide. Specificity, sensitivity, speed, simplicity, cost-effectiveness, and reliability are main criteria for selection of any detection methods.

Since seed serve as means of dispersal and survival, it is critical to test seed before using it as planting material or export purpose. Hence, detection and diagnosis of seed-borne nematodes is a first-line approach for the management of seed-borne nematode diseases of plants. Conventional methods of seed detection assay

include visual examination which helps to understand the sources of seed-borne infections and location within seed tissues, to confirm the occurrence of seed transmission and its mechanisms, and to understand the influence of external biotic and abiotic factors on seed transmission or other phases of the disease cycle, but all have shortcomings ranging from inefficiency to lack of specificity and sensitivity.

Further improvements in the cost and efficiency will eventually allow DNA-based detection systems to replace the vast array of seed detection assays currently employed and provide superior detection capabilities. PCR holds great potential for improving nematode detection in seeds, as it offers several advantages including specificity, sensitivity, rapidity, interpretation, applicability, and ease of implementation.

The feasibility of designing molecular diagnostics tools to distinguish seed-borne nematodes is well established; however, there are still practical constraints. The next stage is validation through routine use of the diagnostics kit to demonstrate whether they are reliable in generating a species-specific signature. Further advancement of technology will lead to improved nematode diagnostics techniques for the identification and containment of quarantine species.

Like other fields in which pathogen detection is critical, seed detection assays must be based on new technologies. However, before adopting these assays, it is critical to rigorously evaluate their applicability, precision, and accuracy in real-world and high-throughput testing of naturally infested seeds. There are many reports of emerging new seed detection assays in the scientific literature; however, only few of them could have developed; hence, little is known about their applicability for routine seed testing. To ensure that these detection assays are efficient, they must be validated in stringent multi-laboratory tests which evaluate their reproducibility and repeatability.

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Part IV

Host–Parasite Interaction During the Development of Seed-Borne Diseases

9 Host-Parasite Interaction During Development of Major Seed-Borne Fungal Diseases

Christian Joseph R. Cumagun

Abstract

Host-parasite interaction using four major seed-borne fungal diseases of cereals as models – *Fusarium* head blight and blast for wheat, bakanae disease of rice, and Philippine downy mildew of maize – is described in this chapter. To establish a reliable method for wheat head blight disease rating, three isolates of *Fusarium graminearum* differing in aggressiveness on susceptible German spring wheat genotype 'Munk' were examined for their upward and downward infection of the floret. The low aggressive isolate was ahead of the symptoms above and below the point of inoculation. The moderately aggressive isolate caused less infection below the point of inoculation compared with the highly aggressive isolate. Latent infection was less frequent and white head symptoms were occasionally observed. High infection below and above the point of inoculation was exhibited by the highly aggressive isolate. There was strong negative correlation between head blight rating and grain weight (r = −83) and number of kernels (r = −94) using 26 isolates of *F. graminearum*. For the wheat-blast fungus interaction, infection of near isogenic isolate of *Pyricularia oryzae* on resistant and susceptible wheat and barley cultivars at seedling stage was observed at the cytological and ultrastructural level. Wheat cultivars St43 and 'Norin 4' (N4) and barley cultivar 'Kwan' (Bar19) were resistant to *P. oryzae* and showed hypersensitive response. Chromatin condensation was observed in St43 and Bar19 indicating an early phase of apoptotic cell response. Significant genotypic variation for aggressiveness of 40 *Fusarium fujikuroi* isolates causing bakanae disease on two rice varieties PSBRc18 and PSBRc54 was observed at seedling stage. The host-pathogen interaction of maize-*Peronosclerospora philippinensis* causing downy mildew of maize at the histopathological level is also discussed.

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9.1 Introduction

Host-parasite interaction denotes relationship taking place between a microorganism and its host. In plant pathology, a microorganism refers to the pathogen which is a living agent whose relationship is parasitic in host plants. Seed-borne fungal pathogens are categorized as internally or externally borne or simply contaminants. The major seed-borne diseases are mostly internally seed-borne infection. *Fusarium* head blight of wheat and wheat blast are considered seed-borne diseases (Xue et al. 2004; Maciel et al. 2014).

This chapter focuses on four important cereal seed-borne pathogens. The study on head blight of wheat dealt with the upward and downward infection of the floret by low, moderately, and highly aggressive isolates of *F. graminearum* and the effect of aggressiveness of 26 isolates on the weight and number of seeds. The wheat blast study provided an in-depth cytological and ultrastructural analysis of the interaction between *P. oryzae* and susceptible and resistant cultivars of wheat. The study on bakanae disease of rice involved the variation in the aggressiveness of *F. fujikuroi* expressed as elongation and stunting of rice seedlings. The host-pathogen interaction of maize-*P. philippinensis* causing downy mildew is reviewed in this chapter mostly from pioneering studies in the Philippines.

9.2 *Fusarium* Head Blight of Wheat

F. graminearum Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch] is the causal agent of devastating diseases of wheat, barley, rye, triticale, and rice worldwide (McMullen et al. 1997; O'Donnell et al. 2000). This fungus causes *Fusarium* head blight (FHB) of wheat which reduces grain yield and quality and produces trichothecene deoxynivalenol (DON); its derivatives, 3-acetyl-DON and 15-acetyl-DON; and nivalenol (NIV) which contaminate grains that are unsafe for human and farm animal consumption (Marasas et al. 1984). Also known as wheat scab, the symptoms on the seeds are characterized as lightweight, shrunken, and discolored with a "tombstone" or chalky white appearance that reduce grain yield and quality. Grouping of *F. graminearum* isolates from a worldwide collection with respect to host or geographical origin was not evident (Cumagun et al. 2006).

Aggressiveness of the pathogen is measured as the quantity of disease induced by a pathogenic isolate on a susceptible host (Van der Plank 1968). In the greenhouse, FHB infection of wheat heads was measured by counting the number of bleached spikelets over the total numbers of spikelets per wheat head. Sudden blighting of the upper spikelets or white head symptoms are usually observed when wheat heads are infected with highly aggressive isolates. In this study, the upward and downward movement of the fungus from the point of inoculation was monitored. This is essential in order to establish a reliable method for disease rating that reflects true infection and thus of immense value in the breeding programs.

Inoculation of susceptible German spring wheat genotype 'Munk' was done at mid-anthesis on four heads per pot by injecting a suspension of approximately 10 µl

with 50,000 conidia/ml of each low, moderately, and highly aggressive isolate of *F. graminearum* by injecting the spore suspension on both sides of the head using a hypodermic syringe needle (0.50 mm gauge) in a controlled plant growth chamber (21 °C day/19 °C night). To provide conducive environment for infection, plants were covered with plastic sheets and incubated for 48 h in the dark (90–100% relative humidity) and then removed and incubated for another 48 h (60–80% relative humidity) under artificial light. After the first 4 days of incubation, plants were transferred to a greenhouse with a mean temperature of 18–20 °C and a day length (artificial light) of 16 h. The number of infected spikelets was counted after 14 days. Plating of individual spikelets on PDA (eight spikelets from one side of the head) by surface sterilization using 1% sodium hypochlorite in 10% ethanol and three changes of sterile distilled water and blotting on dry clean tissue were done.

The low aggressive isolate 10938 is ahead of the symptoms above and below the point of inoculation (Fig. 9.1a). Latent infections which are symptomless could implicate the accuracy of visual rating. White head symptom with empty floret was observed. The moderately aggressive isolate 10954 caused less infection below the point of inoculation compared with the highly aggressive isolate (Fig. 9.1b). Latent infection was less frequent and white head symptoms were occasionally observed. High infection below and above the point of inoculation was exhibited by highly aggressive isolate 10974 (Fig. 9.1c). TeKrony et al. (2001) found that in a susceptible breeding line, infection and movement occurred primarily downward from the point of inoculation to the base of the spike with little movement above the point of inoculation. Seeds in the spikelets above the point of infection are unable to obtain the nutrients and water needed for full development leading to the white head symptom associated with infection prevalent in susceptible cultivars (Schroeder and Christensen 1963). Symptoms spread mainly toward those spikelets positioned below the point of inoculation, and premature ripening is the function of isolate aggressiveness (Malbran et al. 2012).

In another experiment, 26 isolates of *F. graminearum* were inoculated on the same wheat variety and rated for their disease severity expressed as head blight rating. There was a strong negative correlation between head blight rating and grain weight and number of kernels (Fig. 9.2a, b). This study implies that the level of disease rating is an indicator of the quality and quantity of wheat grains to be harvested.

9.3 Wheat Blast

Blast disease is caused by a filamentous ascomycete fungus *P. oryzae* (Couch and Kohn 2002) (teleomorph: *Magnaporthe oryz*ae B.C. Couch) and occurs on rice, wheat, and several genera of grasses (Ou 1985). *P. oryzae* is isolated from approximately 100 gramineous plant species. Wheat blast or 'brusone' is a new field disease of wheat which was first reported in Parana, Brazil, in 1985 causing considerable yield losses (Urashima et al. 1993). Recently, the disease suddenly appeared in Bangladesh for the first time in Asia and caused enormous yield losses in wheat

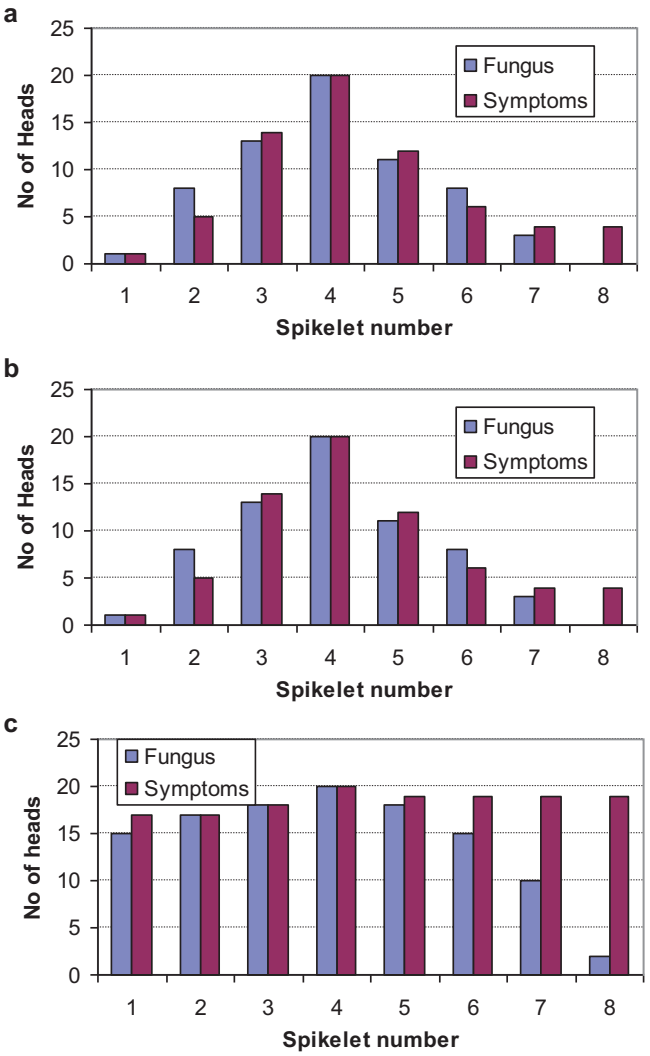

Fig. 9.1 Movement of (**a**) low aggressive 10938, (**b**) moderately aggressive 10954, and (**c**) highly aggressive 10974 isolate within the wheat head 14 days after inoculation. Spikelet number 4 is the point of inoculation at the center of the wheat head

production that could threaten other wheat-producing countries in the region (Callaway 2016). Based on transcriptome sequencing of symptomatic leaf samples collected in Bangladesh, the wheat blast epidemic was caused by wheat-infecting South American lineage from Brazil (Islam et al. 2016). *P. oryzae* infects the wheat rachis with bleached spike above the point of infection producing no grains but remains healthy below this point producing normal grains.

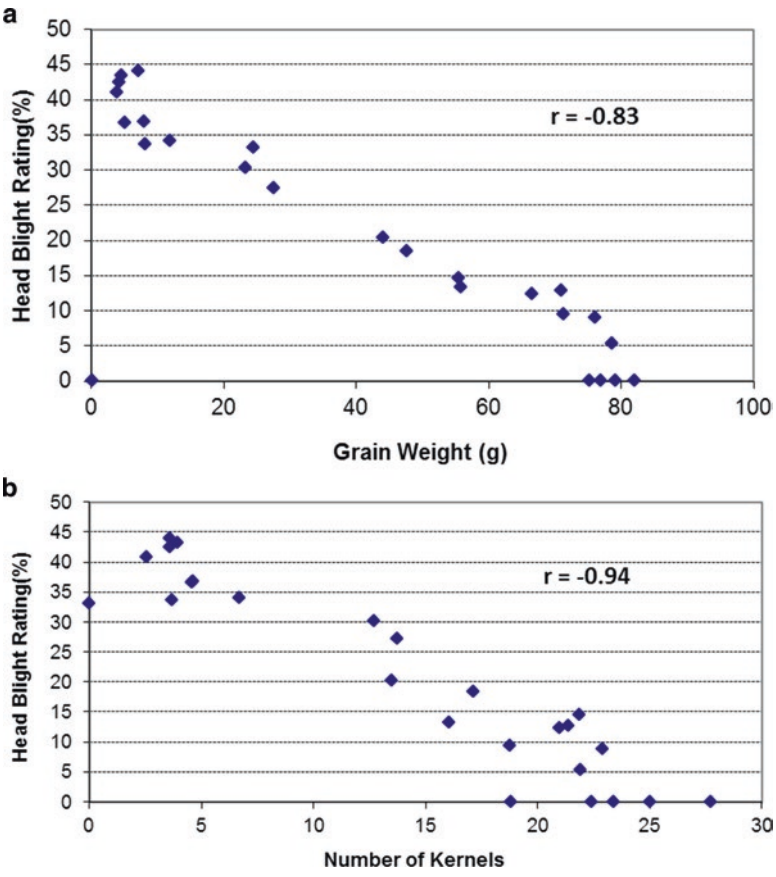

Fig. 9.2 Correlation between head blight rating (%) and (**a**) grain weight (g) and (**b**) number of kernels

Silue et al. (1992) demonstrated that race-cultivar specificity follows the gene-for-gene theory (Flor 1956). The genetic mechanism of host specificity, however, remains to be elucidated. Crossing of fungal strains allows identification of avirulence genes in the same manner as crossing of host species allows identification of resistance genes (Cumagun et al. 2014). To examine the interaction of the *P. oryzae* in two different hosts (wheat and barley), a near isogenic isolate h-31-27 isolated from oats was inoculated under controlled conditions.

The conidial load of 1×10^5–2×10^5 spores/ml (high concentration) was inoculated into resistant and susceptible wheat and barley lines/cultivars. 10 ml of the spore suspension with 0.01% Tween 20 (Nacalai Tesque, Kyoto) were sprayed on the upper surface of the 7-day-old primary leaves in a plastic case with an air compressor. The cases were sealed to maintain high humidity and incubated in darkness for 24 h at 23–24 °C. The test plants were then transferred to a growth chamber at 23–24 °C with a 12-h photoperiod. Symptoms were recorded based on the color and

the size of lesions 4 to 6 days post-inoculation. The size was evaluated using six progressive grades from 0 to 5: 0, no visible evidence of infection; 1, pinpoint spots; 2, small lesions (<1.5 mm); 3, lesions with an intermediate size (<3 mm); 4, large, typical lesions; and 5, complete blighting of leaf blades. These lesions were classified on the basis of their color: brown (B), green (G) and yellow (Y). Infection types were described by both size and color. For example, infection type 2B represents brown lesions with size 2. When mixed-type lesions appeared on a single leaf, the infection type was assessed based on the predominant type. Five plants per line were evaluated for each isolate per replication. The pathogenicity test was repeated at least three times.

For the light microscopy, inoculated primary leaves were harvested 48, 60, and 72 h after inoculation at 20 and 24 °C and boiled in alcoholic lactophenol (lactic acid/phenol/glycerol/distilled water/ethanol = 1:1:1:1:8, vol/vol/vol/vol/vol) for 2 min for fixation. The specimens were mounted on a glass slide in 50% glycerol and observed with an Olympus BX51 microscope (Olympus, Tokyo, Japan). H_2O_2 accumulation was visualized with 3,3-diaminobenzidine (DAB) staining (Rustérucci et al. 2001). The inoculated leaves were dipped in DAB (Nakarai, Tokyo, Japan) solution (1 mg/ml) and vacuum-infiltrated (for 5 min) three times. They were incubated further for 8 h at room temperature in the dark and fixed in a mixture of ethanol and acetic acid (96:4, v/v) overnight. The specimens were mounted on a glass slide in 50% glycerol and observed with the microscope. Cell death was visualized with trypan blue staining (Vogel and Somerville 2000). The inoculated leaves were detached and incubated in alcoholic lactophenol at room temperature overnight. The specimens were boiled in lactophenol solution containing 250 μg/ml trypan blue (Nakarai, Tokyo, Japan) for 2 min, allowed to cool for 1 h, and mounted on a glass slide in 50% glycerol for microscopic observation. For the transmission electron microscopy, wheat cultivars Tat4, N4, and St43 and barley cultivars Bar14, Bar19, and Bar 23 were inoculated with Br48, Br58, and h31–2-7 progeny of *P. oryzae*. Leaf segments (2x 3 mm) were sampled 24, 36, 48, 60, and 72 h after inoculation and prefixed in 2.5% (v/v) glutaraldehyde with 1/5 M phosphate buffer (pH 7.4) at 4 °C for 1 week. After three washings with phosphate buffer, the samples were postfixed with 1% (w/v) osmium tetroxide in phosphate buffer for 1 h and then dehydrated and embedded in Quetol 812 resin. Blocks were cut with a diamond knife on a Reichert-Nissel Ultracut (Leica AG, Vienna, Austria) to obtain ultrathin sections (90 nm), which were then collected on copper grids (200 mesh). Sections were examined and photographed using a Hitachi-7100 (Hitachi, Tokyo, Japan) transmission electron microscope.

Wheat cultivars St43 and N4 and barley cultivar Bar 19 exhibited hypersensitive response and prevented hyphal growth of the near isogenic isolate h31–2-7 of *P. oryzae* (Table 9.1, Fig. 9.3). No hypersensitive response was observed in susceptible wheat cultivar Tat14 and barley cultivar Bar14. Papilla formation, although a form of plant defense reaction, was rarely or not observed in all the wheat and barley cultivars tested. Chromatin condensation was observed in St43 and Bar19 infected with h31–2-7 indicating an early phase of apoptotic cell response (Fig. 9.4). According to Earnshaw (1995), apoptotic cells shrink in size, and the chromatin

Table 9.1 Cytological responses of representative wheat and barley cultivars against *P. oryzae* near isogenic isolate h31–2-7 at 24° C and 60 h post-inoculation

Plant	*Infection type	Near isogenic isolate (h31–2-7)				
		No reaction (A)	Papilla formation (B)	HR (C)	Hyphal growth (D)	HR ratio (C/C + D) × 100
Wheat (St43)	3BG	77.4	0.0	13.2	9.4	58.0
Wheat (Tat14)	5G	40.4	0.0	1.9	57.7	3.0
Wheat (N4)	3BG	38.1	0.0	47.6	14.3	77.0
Barley (Bar23)	5G	100.0	0.0	0.0	0.0	–
Barley (Bar14)	5G	37.0	0.0	0.0	63.0	0.0
Barley (Bar19)	2BG	74.4	2.5	7.7	15.4	33.0

*0=no infection, 1=pinpoint spots, 2=small lesions <1.5mm, 3=intermediate <3mm, 4=large, typical lesions, 5=complete blighting of leaf blades and lesion color (B=brown, G=green)

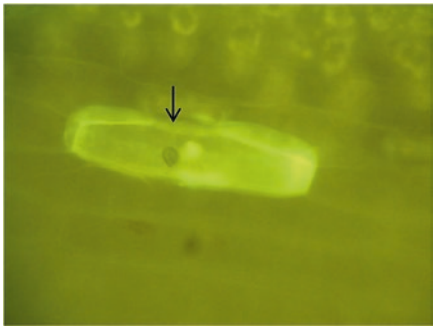

Fig. 9.3 Hypersensitive reaction of barley cultivar 'Kwan' (Bar19) to isolate h31–2-7 of *P. oryzae* after 48 h at 20 °C. Note the appressorium (in arrow) on the epidermal cell

condenses into large granular masses which is considered as a mechanism of programmed cell death not only in animals but also in plants (Heath 1998). Chromatin condensation is part of the death process in oats affected by victorin toxin produced by *Cochliobolus victoriae* (Yao et al. 2001).

9.4 Bakanae Disease of Rice

Bakanae disease caused by *Gibberella fujikuroi* Sawada Wollenworth (teleomorph) *Fusarium fujikuroi* Nirenberg (anamorph) has been known in Japan since 1828. It is widely distributed in all areas that grow rice, particularly in Asia which contribute around 92% of the world's rice production. Furthermore, it is of great importance in countries like Japan, the Philippines, Korea, and India (Ou 1985). "Bakanae," which means "foolish seedlings" (Ou 1985; Webster and Gunnell 1992), is associated with

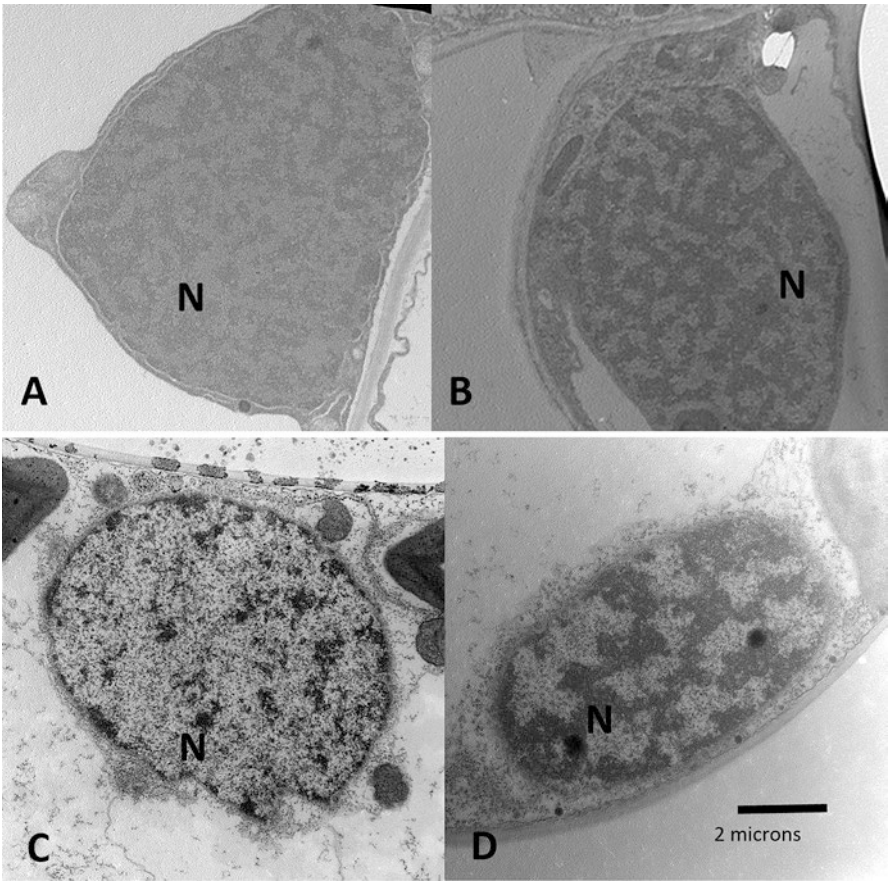

Fig. 9.4 Transmission electron micrographs of nucleus (N) in the mesophyll cells of wheat cultivar St43 (**a**, **b**) and barley cultivar 'Kwan' Bar19 (**c**, **d**) sprayed with wheat isolate Br48 (**a**, **c**) and near isogenic isolate h31–2-7 (**b**, **d**) of *P. oryzae* 60 h post-inoculation

its most conspicuous symptom, abnormal elongation (Fig. 9.5). It was named as "white stalk" in China and "palay lalake," which literally means man rice in the Philippines (Reyes 1939).

Incitant of bakanae is primarily a seed-borne pathogen (Santos 1957); sowing ungerminated seeds in infested soil gives rise to infected seedlings. Seed transmission is the most important mode of transmission than by any other vegetative parts as the pathogen's life span and early possible time of infection are favored. There are five types of seedling symptoms characterized by elongation, normal growth after inoculation, stunted growth after inoculation, stunted growth, and no germination. The occurrence of each symptom varied on the isolate used (Yamanaka and Honkura 1978).

Aggressiveness of 40 *F. fujikuroi* isolates on two rice varieties PSBRc18 and PSBRc54 on a seedling laboratory test to determine the variety-isolate interaction for two trials (=environment) was investigated. The effect of environmental

Fig. 9.5 Bakanae symptoms of abnormal elongation of rice plant infected with *F. fujikuroi*. (Photo courtesy of Dr. Pio Javier, University of the Philippines Los Baños)

variation and isolate-environment interaction on aggressiveness traits (elongation and stunting of seedlings) was also determined. Seeds were sterilized with 10% NaOCl, rinsed with sterile distilled water (SDW) three times, and soaked in SDW for 48 h at 26 °C in the dark. The seeds were transferred to sterile filter papers in Petri plates moistened with SDW and incubated under the same condition mentioned previously. After 2 days, two sprouting seeds were transferred into sterile test tube aseptically. Each isolate was grown in potato dextrose broth (PDB) by inoculating small mycelial disc in a 250 ml Erlenmeyer flask containing 75 ml of the medium. Using a Seitz filter lined with borosilicate microfiber filter (Micro Filtration System, California, USA), liquid cultures were filtrated powered by a vacuum/pressure station (Cole-Parmer International, USA). Culture filtrate of each isolate was pipetted in to the tubes with 0.2 ml per sprout. Tubes were capped and incubated for a week. Seedling overgrowth, as the typical symptom of bakanae disease, served as a parameter for aggressiveness relative to the control which was inoculated with 0.2 ml SDW (Ahmed et al. 1986).

Elongation and stunting of seedlings as measures of aggressiveness varied widely in PSBRc18 and PSBRc54 (Cruz et al. 2013). Majority of the isolates (35) induced similar mean amount of disease as measured by seedling height, while five brought about significant differences in the two varieties across two trials exhibiting variety by isolate interaction. Correlation (r = 0.76) of aggressiveness of 40 isolates of *G. fujikuroi* between the 2 varieties was high, but correlations between 2 trials were moderate (r = 0.44–0.50) suggesting that aggressiveness is affected by the environment. Significant genotypic variation for aggressiveness of isolates ($P = 0.05$) were

observed for both varieties. Repeatabilities for aggressiveness in both trials in PSBRc54 (84.50–89.10) and PSBRc18 (86.50–88.40) were high. Isolate and isolate-environment interaction on the two varieties were also significant ($P = 0.05$) across two trials accounting to 33–35% and 41–43%, respectively, of the total variation. Heritability was medium (0.54–0.55) on PSBRc54 and PSBRc18 due to significant isolate-environment interaction and error. High variation in aggressiveness of *F. fujikuroi* could be due to the ability of the pathogen to undergo sexual reproduction of the isolates including saprophytic phase in soil and seeds.

9.5 Philippine Downy Mildew of Maize

Downy mildew of maize is caused by the oomycete *Peronosclerospora philippinensis* (W. Weston) C.G. Shaw. The pathogen was first described by Weston (1920) as *Sclerospora philippinensis* W. Weston. The disease symptom is characterized by the typical chlorotic streaks running parallel to the leaf veins (Fig. 9.6) as well as mass of downy whitish conidiophores and conidia covering the underside of the leaves.

These conidiophores are always produced during the night when dew deposits and conidia are dispersed from it by air during the midnight and up till early in the morning during which the remaining conidiophores desiccate. Mycelial hyphae grow intercellularly in all parts of the maize plant except the roots (McTaggart and Cumagun 2007). During penetration, spores germinate on the leaf surface less than 1 h after inoculation after which long and short germ tubes are produced. Penetration of the stomata with and without appressoria takes approximately 2 h after inoculation (Dalmacio and Exconde 1969).

Two types of hyphae, namely, the long, slender hyphae that lie alongside the vascular bundles in the leaves and stems; and the lobed, crooked hyphae between the mesophyll cells of the leaves, produced haustoria, but they were most pronounced on the latter. Conidiophores coming out of the stomata invade the chlorotic sections on the leaf surface, husks, glumes, sheaths, and tassel rachis (Weston 1920;

Fig. 9.6 Characteristic symptoms of downy mildew of maize showing chlorotic streaks running parallel to the veins

Dalmacio and Exconde 1969). Hyphae of *P. philippinensis* grew downward through the leaf sheath, and 5 to 6 days after inoculation, they invade the stem. From the stem, the fungus moves both upward and downward where the latter was restricted by more or less mature tissues of the upper part of the internodes.

The first symptom is characterized by chlorotic areas at the base of the leaf and until they gradually spread on all the leaves with the youngest leaf becoming completely chlorotic (Fig. 9.6) (Dalmacio and Exconde 1969). Parenchymatous tissues of the seeds become infected, and later on the pericarp, leaving the embryo and endosperm free of mycelium (Exconde 1976). According to Advincula and Exconde (1976), *P. philippinensis* is transmitted through the seeds. Both local and systemic symptoms resulting from seed infection are produced. Local symptom appeared in the second and third leaves in the form of chlorotic stripes as early as 12 days after sowing, whereas systemic symptoms appeared 9 days after planting in the form of chlorosis of the first true leaf.

9.6 Conclusion

Host-pathogen interaction of the four pathosystems, wheat-*Fusarium*, wheat-*Pyricularia*, rice-*Fusarium*, and maize-*Peronosclerospora*, is discussed in this chapter, of which the last pathosystem was generated from the secondary data by pioneering works in the Philippines. The first three pathosystems were produced from primary data by the author using mostly phenotypic tools along ultramicroscopical analysis as in the case of wheat-*Pyricularia* pathosystem. Future studies of host-pathogen interaction at the molecular level are needed particularly in dealing the less studied diseases such as bakanae disease of rice and Philippine downy mildew of maize.

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Host-Parasite Interaction during Development of Major Seed-Borne Bacterial Diseases

10

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Abstract

Parasitic species demonstrate a wide range of population structures and life cycle plan, including various transmission modes, life cycle complication, survivability, and dispersal ability with and without the presence of their hosts. A prominent feature of hosts and parasites is based on their genetics which can be regulated by coevolution. Infections measured under laboratory conditions have shown that the environment in which hosts and parasites interact might substantially affect the strength and specificity of selection. An effective defense response is the precursor of evolution in plant immunity which restricts the potential onset of disease by microbial pathogens (parasites). In plants, the primary immune response, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), is one of the best examples of evolution to acknowledge general characteristics of microbial pathogens. Such type of coevolution was manifested in host-parasite interactions, but the knowledge is very less. The behavior of parasite and environmental factors also affects the host-parasite interactions. The environmental conditions such as moisture content, temperature, wind velocity, and availability of food are major factors in host-parasite interaction. The environment provides a suitable condition for the establishment of host and their parasite. In this book chapter, we are focusing on coevolution, environmental effect, and specificity during host-parasite interactions.

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10.1 Introduction

Plants always interact with various microbes without having a somatically adaptive immune system against them. However, in presence of these microbes, the plant populations do not frequently subject to devastating disease epidemics. Disease development through seed-borne inoculum is the best result of host (plant) and parasite (pathogen) interaction in the nature. The study of these interactions is known as epidemiology. Seed-borne diseases are one of the main factors for the great economic loss in agriculture in various forms. Losses might start early from reduced germination, and it may include inadequate seedling vigor due to immature seed and crop damage at the level of seedling growth, development to harvest, and storage. In many instances, the losses occur due to previous crop produced from infested seeds. In that case the pathogens survive in soil and on debris of crop and weeds which results in further attack on subsequent susceptible crops. Sometimes, the inoculum is available in very poor quantity and unable to infect plants. Therefore, it takes too much time to accomplish a detectable level of disease in the crops (Sheppard 1998). To overcome these losses, plants need to show disease resistance against various seed-borne diseases. It is a very complex procedure that provides various potential barriers to inhibit the pathogen invasion. The disease resistance (R) genes are one of the main defense mechanisms which activate the defense responses, namely, localized cell death during the encounter with pathogenic microbes carrying respective avirulence genes (Dangl and Jones 2001; Allen et al. 2004).

The coevolution in host-parasite interaction is reciprocal natural selection between host resistance and parasite infection potential (Thompson 1999). The hypothesis proposed that the reciprocal natural selection totally relies on frequency (Bell and Smith 1987; Hamilton et al. 1990), in which the parasites are picked out to minimize the common host's resistance, and accordingly the hosts having rare resistance genes are favored for selection (Carius et al. 2001). The other interaction of nearby species provides crude materials for coevolutionary change (Thompson 1999). The local host-parasite interactions are also dependent on adjacent populations because these populations have different strategies for their resistance and virulence genes. Therefore, the new resistance and virulence genes may enter in local populations through gene flow (Gandon et al. 1998). Local extinctions, founder effects, and genetic drift also have the ability to determine the interactions and lead to shape a large-scale picture (Thrall and Burdon 1997; Burdon and Thrall 1999; Thompson 1999; Carius et al. 2001).

10.1.1 *Pseudomonas syringae* pv. *tomato*

The bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato* is generally found growing epiphytically. The host range of the pathogen is very broad even though its population falls in absence of susceptible hosts (Preston 2000). Several economically important diseases are caused by *Pseudomonas syringae* in number of

its host plants. *P. syringae* pv. *tomato* can survive on seeds of tomato inside the seed cavities and lead to the infection of whole tomato fruit (Devash et al. 1980). Generally infested seeds develop visible symptoms and sometimes remain symptomless. However, infested plants growing in high relative humidity develop high amount of pathogen population and serve as source of infection (McCarter et al. 1983). A number of reports suggest that it is a seed-borne disease (Bashan et al. 1982). Each strain of *P. syringae* has some sort of host specificity, and only few of the plant species get infected, sometimes only few cultivars of a plant species (Xin and He 2013). Moisture, cool condition, and temperature around 12–25 °C favors the disease spread; however, this can depend on pathovar of the pathogen. The disease is seed-borne, and rain splashes are majorly involved in its dispersal to other noninfected plants (Hirano and Upper 1990). Though being a plant pathogen, *P. syringae* can facilitate itself as saprophyte around the phyllosphere in the unfavorable condition (Hirano and Upper 2000). *P. syringae* strains, which are saprophytic in nature, have the ability to act as biocontrol agent in postharvest rot disease (Janisiewicz and Marchi 1992). The ability of pathogen to cause disease can be divided into a number of categories such as overcoming the host resistance, ability to go inside the plant, forming biofilm, and producing some proteins having ice-nucleating properties (Ichinose et al. 2013). *P. syringae* is planktonic in nature, able to invade the plant, and with the help of pili and flagella can move toward its host. The main insertion site for *P. syringae* is through natural opening sites and wounds, and it is also able to break the cell wall. The way of movement of bacteria toward the plant is not well-studied, but some of the studies showed the chemotactic movement toward the plant and caused the infection (Ichinose et al. 2013). *P. syringae* isolates adopt type III secretion system (T3SS) for its virulence. The effector proteins of T3SS are a major cause to modify the host immune system in favor of the pathogen for infection. The hrp gene clusters are the major group of T3SS effector proteins and codes Hrp secretion apparatus. Some other ways like production of phytotoxins such as coronatine of pathovar Pto and Pg have the ability to suppress the host immunity and invade the host plant (Ichinose et al. 2013).

Certain polysaccharides produced by *P. syringae* causes the adherence of the pathogen to the plant cell surface. The communicating phenomenon 'quorum sensing' is reported for the bacteria to communicate the other bacterial cells nearby. When the level of quorum sensing molecules crosses the threshold, the bacteria starts biofilm formation and expression of genes related to virulence. Some highly viscous compounds such as DNA and polysaccharides are secreted by *P. syringae*, which create the protective environment for growth of the pathogen (Ichinose et al. 2013). *P. syringae* majorly causes cell wall damage by chilling in plants greater than any other organisms or minerals. The plants which are devoid of any antifreeze proteins are majorly damaged by −4 to −12 °C temperature, and in this condition, water remains as supercooled liquid in plants. *P. syringae* have ability to cause freezing of water on slightly higher temperature, i.e., −1.8 °C (28.8 °F) (Maki et al. 1974), but ice nucleation is generally reported at lower temperature (−8 °C). The epithelial cells are injured by freezing, which causes nutrient availability to bacteria from plants. Certain ice-nucleating genes coding ice-nucleating proteins

translocated to bacterial membrane and act as nuclei for ice formation (Fall and Wolber 1995). Two races of the *P. syringae* in tomato (0 and 1) have been described around the world (Lawton and MacNeill 1986). Due to development of resistant tomato varieties, a selection pressure created on race 0 causes appearance of new race 1 even on heterozygous nature of Pto gene in tomato (Buonaurio et al. 1996). The appearance of new race of *P. syringae* due to newer resistant varieties of tomato plant is a well-known complicated mechanism of plant-pathogen interaction (Mew et al. 1992). The development of newer race is advantageous for the pathogen (Pohronezny et al. 1992). The selection pressure increases due to introduction of newer imported resistant varieties and hybrids. Certain countries like the Balkan Peninsula and the Mediterranean, where tomato is grown intensively, observe the seed-borne nature of the *P. syringae*. However, unwillingly introduction of the pathogen with imported infected seeds cannot be excluded for the newer race development as it was reported earlier in other countries. The host plant resistance increases due to introduction of resistance genes in plants to improve control of *P. syringae* (Milijašević et al. 2009). A report found that ABA signaling pathway is the major target of effectors secreted by pathogen. Modulation of PP2C gene expression affected hypersensitive reaction toward ABA, which is otherwise helpful to bacterial multiplication. ABA level is found to be increased during bacterial colonization. However, exogenous application of ABA enhances susceptibility reaction. As per the data shown by de Torres-Zabala et al. (2007), the virulence strategy of pathogen due to presence of effector protein leads to suppresion in host defense responses.

10.1.2 *Xanthomonas campestris* pv. *campestris*

The strains of genus *Xanthomonas* infect up to 124 monocot and 268 dicot plants and create severe economic damage in the warm and humid region (Chan and Goodwin 1999). The black rot disease is caused by *Xanthomonas campestris* pv. *campestris* (Xcc) and also considered as one of the most devastating diseases of crucifers worldwide infecting most varieties of brassicas including broccoli, cabbage, kale, oilseed rape, cauliflower, turnip, radish, and mustard along with model plant *Arabidopsis thaliana* (Williams 1980). For all these conditions, Xcc-infected seeds are the basic source of inoculum. Throughout the germination epicotyl of seedling gets infected (Alvarez 2000) and the developing cotyledons became black at the margin, wilt, and fall down. The pathogen strides out through vascular system of the plants to young stem and leaves. The disease appears in V-shaped chlorotic to necrotic lesions and extends from the margin of leaves. During the humid environment, the bacteria oozes out and forms a droplet by the process of guttation through hydathodes and these droplets may spread through wind, rain, and/or through mechanical damage to their neighboring plants. The pathogen (Xcc) gains entry through the wounded leaves and plant roots due to insect damage or through hydathodes which is natural route of infection. Sometimes but rarely the infection also occurs through stomata. The hydathodes provide a straight path for pathogen to enter from leaf margin to vascular system of plant and hence systemic infection in

host. Attack through the suture vein leads to the formation of Xcc-infected seed. Xcc has the ability to survive in plant debris present in soil up to 2 years, but it hardly remains in free soil up to 6 months (Alvarez 2000), and it serves as a source of secondary inoculum. In a significant development, bean flowers inoculated with *X. campestris* pv. *campestris* led to the production of higher level of infested seeds and are also carried efficiently to seedlings of bean plants (during incompatible interaction) (Darrasse et al. 2010). This kind of floral pathway might allow the production of contaminated seeds by cohort of different bacteria and also sometimes includes biocontrol agents (Fessehaie and Walcott 2005). Similarly, the type III secretion system mutants of *X. citri* pv. *phaseoli* var. *fuscans* leads to the production of infested seeds through the entry from floral pathway and supports the previous hypothesis strongly (Darsonval et al. 2008). However, the contrasting result was found when these mutants were applied through vascular system of plants and no infested seeds were found, as found for wild-type strain.

The interactions between seeds or seedlings of the plants with the bacteria allowed multiplication of bacteria without any negative result on plants during the commensal interaction. During the compatible interactions, the seed-borne xanthomonads colonize on the surface of seedling and have no negative impact on early endophytic development (Gilbertson and Maxwell 1992). The bacteria do not require a molecular crosstalk with plants to colonize efficiently because rich amount of nutrients are available during germinating seeds and seedlings (Nelson 2004). Practically, the similar results were observed for *X. campestris* pv. *campestris*, *E. coli*, and *X. citri* pv. *phaseoli* var. *fuscans* in bean seed imbibition to get 14-day-old seedlings representing incompatible, null interactions and compatible interactions, respectively. Apart from this the bacterial colonization also does not need a functional T3SS in spermosphere, suggesting that nutrients are not major limiting factors and T3SS genes are not induced in the presence of nutrient-rich medium (Valls et al. 2006). This result was reversed in the phyllosphere as the nutrient-rich medium is a limiting factor (Mercier and Lindow 2000). The bacteria need the expression of T3SS gene to colonize efficiently in phyllosphere. Both the results suggest that for effective colonization on seedling, bacteria need different strains than phyllosphere multiplication, and these environmental parameters are also different for bacteria to adopt. Certain special interactions between bean plants and bacteria *X. citri* pv. *phaseoli* var. *fuscans* have occurred during the multiplication in phyllosphere (Darsonval et al. 2009), where nutrient availability is a major limiting factor for bacterial colonization. Additionally, the *X. citri* pv. *phaseoli* var. *fuscans* downregulate the expression of PR-3 gene in leaves indicating the suppression of plant defense. This is the sequential action of T3SS effectors to inhibit the defense response stimulated by PAMPs during compatible interactions (Mishina and Zeier 2007). During the incompatible interaction, no enhanced defense response induction was observed by *X. campestris* pv. *campestris* in bean seedlings. Therefore, these results indicate that defense responses are induced in early-stage plantlets when *X. campestris* pv. *campestris* and acibenzolar-S-methyl were applied on seedling and leaves as inoculum. The similar defense response induction was also observed earlier in melon and cowpea seedling (Buzi et al. 2004). This clearly

showed that wild-type strain *X. campestris* pv. *campestris* induces defense responses following the infiltration inoculation of seedlings, while the mutant in T3SS *X. campestris* pv. *campestris* is unable to induce defense response.

10.2 Differentiation of Molecular Pattern for Microbe Identification

Similar to animal system, plants also have the innate immune response which is turned on subsequently after the identification of invading microbes (Nürnberger et al. 2004; Akira et al. 2006; Spoel and Dong 2012). Some of these microbes have no targeted effect on plant growth and development. However, among them huge variety of microbes present in plant's microbiome are either beneficial or pathogenic (Berendsen et al. 2012). Beneficial interaction with microbes provides root colonization, plant growth promotion, and yield and also induces plant defense either directly or indirectly. Since the beneficial microflora were initially thought to be alien organisms that modify the immune system of plants for a successful establishment of mutual relationship with their host (Zamioudis and Pieterse, 2012), it is important for the plants to not only distinguish the microbes but also to possess the ability to differentiate them as good or bad and thus react accordingly. This process is very important during the plant-microbe interaction for their better development and protection, and also it needs to maximize the same. Similarly, the reverse action is needed for microbe to regulate host immune system to avoid an array of effective defense according to their interaction and relationship (Pel and Pieterse 2013).

Identification and differentiation between self and foreign molecule is a very crucial first step to initiate the effective immune response through pattern recognition receptors (PRRs) in the host plant cells. The PRRs identify these microbe-associated molecular patterns (MAMPs), which were earlier known as pathogen-associated molecular patterns (PAMPs) (Boller and Felix 2009). After recognition of PAMPs through the plant's PRRs, plants respond with an enhanced immune response known as PAMP-triggered immunity (PTI). The PTI is a first line of defense in plants effective against their various non-accommodated pathogens (Jones and Dangl 2006). The most prominent examples of MAMP perception in plants are recognition of conserved RNA-binding motif of bacterial cold-shock proteins (Felix and Boller 2003) and 17 amino acid-conserved domain of the Ax21 protein of *Xanthomonas* through Xa21 receptor of rice (Song et al. 1995; Lee et al. 2009). Pathogens are able to escape themselves from recognition through their evolutionary adaptations of MAMPs. Apart from this, pathogens also have the capability to secrete certain proteins known as effector proteins which modulate the plant's defense mechanisms and make them susceptible for pathogenesis. The type III secreted proteins AvrPto and AvrPtoB of *P. syringae* are well-studied case of these effector proteins. AvrPto usually binds with kinase domain of EFR, FLS2, RLKs, BAK1, and CERK1, while AvrPtoB binds FLS2 and degrades them. Hence the MAMP signaling is blocked (Göhre and Robatzek 2008; Shan et al. 2008; Xiang et al. 2008; Gimenez-Ibanez et al. 2009). In the course of coevolution

between hosts and pathogens, the plants develop resistance (R) proteins for the recognition of particular effectors of pathogens leading toward secondary immune response known as effector-triggered immunity (ETI) (Fig. 10.1). Hence, the final result of the combat between hosts and pathogens is known as zigzag model (Jones and Dangl 2006). It totally depends on balance battle between the capability of pathogens to inhibit the host's immune response and the capability of host plant to identify the pathogens and activate an array of effective defense accordingly. The recent studies discovered proteins other than effector proteins that inhibit the primary immune response of infected tissue of host plant. These proteins are present in both fungal and bacterial pathogens which escape the identification of MAMPs and interfere before microbe recognition by host plants. In addition to this, certain microbes modify their MAMPs structure to suppress the MAMPs identification. Bacteria also adopt similar escape plan from recognition by the host plants. During the search of TLR5 signaling of antagonism, type I secreted alkaline protease AprA was recognized in the supernatant of *Pseudomonas aeruginosa* (Bardoel et al. 2011). The zinc metalloprotease AprA belongs to the serralysin family in Gram-negative bacteria, and most of the members of this family are virulence factors (Stocker et al. 1995). In human cells, the addition of AprA before flagellin treatment results in weaker induced immune responses, while addition of higher

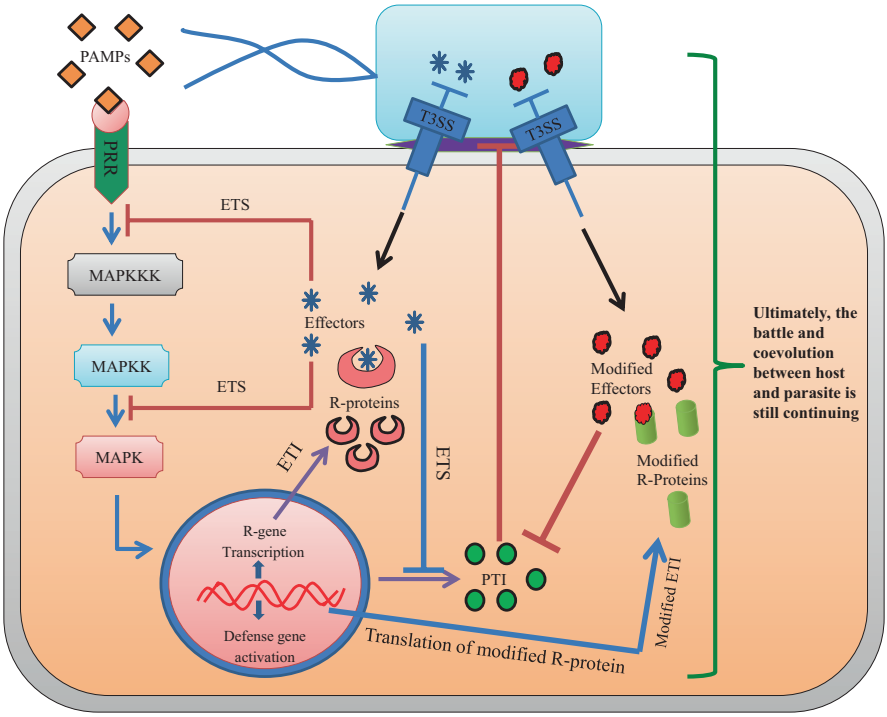

Fig. 10.1 Diagrammatic representation of battle between host and parasite which leads the coevolution in both

concentration of AprA shows no flagellin-induced immune responses. Similarly, in plants the treatment of *P. aeruginosa* aprA mutants to *A. thaliana* shows faster stomatal closure to that of induced stomatal closure through wild-type bacteria. Thus, flagellin degradation by AprA enhances the ability of *P. aeruginosa* to avoid the recognition by host immune system in both human and plants (Bardoel et al. 2011). Although various MAMPs are widely distributed and conserved in pathogenic microbes, beneficial microbes also have similar MAMPs-like pathogens. Therefore, it is important for plants to discriminate between beneficial and pathogenic microbes to get benefitted in presence of beneficial microbes. Studies are exploring good evidences which proposed that beneficial microbes are also recognized as potential invaders at the beginning and plant activate their immune response (Zamioudis and Pieterse 2012). An example of *Rhizobium*, a beneficial bacterium that forms symbiotic interaction with leguminous plants where they form nodules to reside and fix atmospheric nitrogen, is well-known. Initially, the plants recognize them as a pathogen, resulting in the stimulation and activation of defense gene expression (Kouchi et al. 2004; Lohar et al. 2006; Zamioudis and Pieterse 2012). For the successful symbiotic interaction, rhizobia involve themselves to avoid recognition in a similar fashion like pathogen. *Sinorhizobium meliloti* and *Mesorhizobium loti* bring out flagellin molecules that do not induce defense responses of plants (Felix et al. 1999; Lopez-Gomez et al. 2012). This recent study supports the importance of avoiding recognition for beneficial microorganisms. Apart from this, in various *Rhizobium* species, homologues of AprA are also found to prevent the recognition. Further, throughout the afterward stages of interaction, rhizobial colonization in plants and downregulation of expression of defense-related genes indicate that *Rhizobium* bacteria become successful to reduce host plants' defense responses (El Yahyaoui et al. 2004; Kouchi et al. 2004; Lohar et al. 2006; Moreau et al. 2011). LPS is one of the bacterial molecules from *S. meliloti* having the capability to reduce the defense response of the host plant. The cell culture of plant treated with LPS of *S. meliloti* initiates a much diluted defense response in host plants *Medicago sativa*. In another experiment, the simultaneous application of LPS of *S. meliloti* and defense elicitors from yeast shows suppressed early- and late-induced defense responses in *M. sativa*. This restrictive capability of LPS appears very narrow to *S. meliloti-M. sativa* association because nonhost plants show usual response toward the LPS of *S. meliloti* (Albus et al. 2001; Scheidle et al. 2005; Tellstrom et al. 2007). Plant growth-promoting rhizobacteria (PGPRs) form nonsymbiotic interaction with plants and enhance plant growth (Lugtenberg and Kamilova 2009). In similar trend of rhizobia, PGPRs also stimulate PTI response in plants (Bakker et al. 2007; Van Wees et al. 2008). Thus, PGPRs should reduce the degree of identification by host plants for insignificant stimulation of host defense arsenal (Millet et al. 2010). Phase variation is one of the possible strategies for PGPRs to reduce the recognition during root colonization of host plants. The reversible switching of two phenotypic stages in bacteria as per requirement according to environmental condition is known as phase variation (Davidson and Surette 2008; Van der Woude 2011). Regarding the soft interactions in between the plant roots and soil-borne mutualistic microbes, lots of more

mutualistic microbial effectors are still unexplored that have the capability to modulate the immune response of host for successful interaction of plants and beneficial microbes. In the last few years, the researcher proved that hormone-regulated network of signaling in plants is the first aim of both pathogenic and beneficial microbe (Jacobs et al. 2011; Kloppholz et al. 2011; Plett et al. 2011; Pieterse et al. 2012; Zamioudis and Pieterse 2012).

10.2.1 Plant Defense

The evolution of immune system in plant cell reaches to high level that is able to prevent the attack of the pathogenic microorganism. The common property pathogenic microorganisms are firstly detected by the primary immune response known as PAMP-triggered immunity (PTI) by the plant cell. Pathogen acquires some protein through coevolution of host-microbes interaction to suppress the PAMP-triggered immunity (PTI) and enhance the growth of pathogen and disease. To monitor the pathogen effector protein either directly or indirectly, plant cell acquired surveillance proteins (R proteins) (Chisholm et al. 2006). Pathogen has to overcome three defense systems to infect the plant: (1) shaped physical barriers, (2), a cell outer surface investigating system that finds conserved molecules of pathogen, (3) and a surveillance system that detects effector molecule that pushes into intracellular host by pathogenic organism.

Bacterial pathogen defeats the primary layer either through natural opening or wounds or by the help of enzymatic activity through damaging the layer of plant cell surface. The second layer of defense system is overcome by injecting effectors protein. The third layer is overcome either by modifying or eliminating existing effectors or by developing new effector. Wilts, galls, specks, spots, cankers, and chlorosis (yellowing) are produced by pathogenic bacterial infection during disease. For example, water and nutrient supply is blocked during wilt causing bacterial infection in vascular tissue. Recently, *Xylella fastidiosa* are most studied plant pathogenic bacteria responsible for major decrease in the production of grapes in California. To break the barriers, some plant pathogenic microbes introduced a wide range of extracellular virulence components like cutin-degrading enzymes, cell wall-degrading enzymes, etc. These enzymes are released by a type II secretary path. Cell wall-degrading enzymes cellulases, pectinases, and endoglucanases are also released via the same process. These enzymes are important for causing soft rot in plant cell by bacteria specially the *Erwinia* genus (Ade et al. 2007).

The plants initiate signal-transduction cascades after the sensation of PAMPs that start basal defenses. The deposition of callose and silicone for cell wall strenghtening, production of ethylene and reactive oxygen species (ROS), trancriptional activation of different defence gene carrying PR-genes, and post-transcriptional inhibition of auxin signaling are the various mechanisms that come under basal defence responses. The bacterial flagellin is a well-described molecule that induces basal defense in host plants. The bacterial flagellin (flg22) is a 22 amino acid long peptide which activates basal defense response in plants, namely, generation of ROS

and alkalinization of the extracellular matrix (Felix et al. 1999). In *Arabidopsis* the basal response induced by flg22 also includes closure of stomata to inhibit the bacterial entrance. In *Arabidopsis*, the flg22 is recognized by a transmembrane receptor kinase PRR FLS2. The fls2 mutant *Arabidopsis* plants are more sensitive to *Pseudomonas syringae* pv. *tomato* (PstDC3000) infection, when bacteria are used on leaf surface for infection in comparison to infiltration of bacteria into intercellular spaces of leaf (Zipfel et al. 2004). These experiments suggest that FLS2 is one of the factors responsible for the activation of initial defense responses and inhibits the entry of bacterial pathogen *Pseudomonas syringae* pv. *tomato* into plants. The recent reports also indicate that flg22 inhibit the expression of mRNA of certain auxin receptor genes (Navarro et al. 2006). It is quite interesting that study showing downregulation was proved through micro (RNA) miR393 via posttranscriptional modifications. The flg22 enhances the expression of miR393, and downstream action of miR393 results in the degradation of mRNAs responsible for the action of auxin receptor genes, AFB2, AFB3, and TIR1. *P. syringae* is well-known for the production of auxin for their own benefit. The inhibition of auxin signaling leads to basal resistance because overexpression of auxin makes it more sensitive to *P. syringae* (Navarro et al. 2006). Apart from flagellin, the elongation factor Tu (EF-Tu) present in profuse amount in increasing bacterial population also functions as activator of basal defense response in plants (Kunze et al. 2004). EF-Tu is 43 kDa protein and 18 amino acids present at N-terminus (elf18) of this, also able to induce basal defense response by itself. In *Arabidopsis*, elf18 is recognized by EFTu receptor (EFR), a receptor-like kinase (RLK). The intracellular kinase domain and extracellular leucine-rich repeats (LRR) domain of EFR has structural similarities with FLS2 (Zipfel et al. 2006). Hence, it is clear that the perception and recognition of bacterial pathogens by plants and subsequent initiation of basal defense is not relying on a single factor. The basal defense response can also be induced by various other factors. The plants using multiple PAMPs during the perception of microbes are totally dependent on the recognition of pathogens and activation of defense response. The initiation of defense responses in plants is triggered by the perception of PAMPs; therefore, it is known as PAMP-triggered immunity (PTI) (Jones and Dangl 2006).

The pathogenic bacteria adopt one strategy to overcome the defense of plants which is the secretion of coronatine (COR), a small molecule and virulence factor that mimics as jasmonic acid hormone secreted by plants (Melotto et al. 2006). COR-mediated inhibition of stomatal closure is induced by suppression of PAMP-stimulated abscisic acid (ABA) signaling within guard cell. The bacterial mutant lacking coronatine shows diminished virulence to that of wild-type bacteria during the inoculation onto the plant leaf surface for infection. The colonization on host plants and inhibition of basal defense response through particular effector proteins is another strategy for causing disease also reported in bacterial pathogens. These effectors are normally translocated straight into the host cell from bacterial cell employing type III secretion system (T3SS), most common in *Ralstonia solanacearum*, *Erwinia*, all *Pseudomonas* pathovars, and *Xanthomonas* (Hueck 1998).

When T3SS effectors minimize or stop basal defense signaling, plants adopt secondary defense response and recognize the existing T3SS effectors within the plant cell, a best example of coevolution (Fig. 10.1). This recognition system uses the receptors present inside the plant cell, encoded by resistance (R) gene. Most of the R proteins present in plants include nucleotide-binding site and leucine-rich repeat (NBS-LRR). The NBS-LRR proteins regulate resistance against majority of plant pathogens. Stimulation of R protein by effector protein of pathogen triggers the initiation of programmed cell death (PCD) at the site of pathogen infection and surrounding tissues in plants. This R protein-regulated resistance is known as effector-triggered immunity (ETI), and localized PCD is known as hypersensitive response (HR). Apart from the stimulation of PCD, R protein-regulated resistance also includes the generation nitric oxide (NO) and reactive oxygen species (ROS) which further have the capability of signaling molecule and antimicrobial activity. ROS along with NO induce the transcriptional activation of defense genes and HR in plants. The superoxide anion (O_2^-) and NO in combination form highly toxic peroxynitrite ($ONOO^-$), which participates either directly or indirectly in death of pathogens and host plant's cell (Saito et al. 2006). Here the pathogens evolve themselves for self-defense by secreting various enzymes, free radical scavengers, and production of antioxidants to fight against abovementioned plant defense molecules. An emerging new theory explores the capability of certain R proteins to trigger gene silencing through small interfering RNA (siRNA) in pathogens. The RPS2 is a R protein in *Arabidopsis* which has been experimentally proven to enhance siRNA nat-siRNAATGB2 to silence the PPRL gene (Katiyar-Agarwal et al. 2006). The RPS2-mediated disease resistance is diminished by overexpression of PPRL gene in *P. syringae*. Therefore, it seems that the signaling pathway mediated by RPS2 downregulates the expression of PPRL, and full elicitation of RPS2-mediated signaling needs the degradation of PPRL mRNA through siRNA. Now, the bacterial pathogens had any coevolution to enhance their pathogenesis against siRNA, and miRNA-regulated resistance is still yet to be explored. The recognition of bacterial effector molecule by plant R proteins at molecular level might be either direct or indirect. The model representing direct recognition is also known as "ligand-receptor model." This model hypothesized that the R proteins act as receptor and bind to ligand effector proteins of the pathogen directly (Deslandes et al. 2003). The model representing indirect recognition is known as "guard model" which shows the spots of changes occur in host plant protein by effectors. This model is very common in bacterial pathogens and often has been explored for effector molecules like AvrPtoB, AvrRpt2, AvrRpm1/AvrB, and AvrPphB in *P. syringae* (Kim et al. 2002; Mackey et al. 2002; Axtell and Staskawicz 2003; Ade et al. 2007).

Indirect recognition model is supported by the interaction of two R proteins RPM1 and RPS2 with RIN 4 protein in *Arabidopsis*. Especially RPM1 interacts with the phosphorylated form of RIN4 induced by the interaction with AvrRpm1 and AvrB effector proteins of the *P. syringae* (Mackey et al. 2002). Additionally, indirect recognition model can also be elaborated with the interaction of RPS5 protein of *Arabidopsis* with PSB1 protein. PSB1 protein is also part of *Arabidopsis*

itself cleaved by a cysteine protease effector AvrPphB of *P. syringae*. Cleaved product of PBS1 is recognized by R protein RPS5 leading to activation of HR (Ade et al. 2007).

These traits related to resistance are introduced into the plants through resistance breeding. Pathogens also have the mechanism to overcome such type of resistance by developing new pathogenic strains that leads to the next round of resistance breeding. This everlasting battle during host-pathogen interaction has received much more attention from researchers. Recently, various studies provided the insight lying behind this phenomenon. Similar to suppression of PTI, bacteria also have developed the mechanism to suppress ETI. The development of a new effector is one of these mechanisms to suppress R gene-mediated hypersensitive response related to disease resistance. Hop-AB1 and HopZ3 are two effector proteins of *P. syringae* pv. *syringae* which are able to suppress programmed cell death initiated by some other effector molecules in *N. benthamiana* (Vinatzer et al. 2006). HopAB1 and HopZ3 mutants from *P. syringae* pv. *syringae* restores its capability to provoke the HR. Similarly, some other effectors from Pst DC3000 such as AvrPtoB, HopPtoE, AvrPphEPto, AvrPpiB1Pto, and HopPtoF are capable to retard effector-triggered programmed cell death in tobacco. *P. syringae* pv. *phaseolicola* also has the ability to suppress the effector-triggered PCD via altered effector in bean in a cultivar-specific manner. Recently immunity-associated protein AtMIN7 was found to be destroyed by an effector HopM1 from *P. syringae* leading to the development of disease in the model plant *Arabidopsis* (Nomura et al. 2005). Coevolution of Avr gene in context to host R gene defines the suppression of effector-triggered HR, an important component of ETI leading to development of disease by bacterial pathogen *P. syringae.*

10.2.2 Host Specificity

Host specificity of pathogens basically depends upon growth, colonization, and infection ability to their respective hosts (Kirzinger and Stavrinides 2012). Bacterial pathogens show various mechanisms of host specificity by modulating their genome-like duplication, point mutation and horizontal gene transfer (HGT), etc. Certain bacterial pathogens have very broad host range, and the earlier studies of symbiotic bacteria determine a single regulatory gene for the host specificity (Mandel et al. 2009). Divergent gene expression of bacterial single regulatory gene expands the successful colonization on various host species. The regulation of gene expression might be the key factor for host specificity in bacterial pathogens contributing to the emergence of new disease due to development of new pathogenic strains. The molecular interactions of hosts and pathogens are the key factors that demonstrate host specificity in bacterial pathogens (Pan et al. 2014). Very small changes in host-pathogen interactions might cause great modification in host range and state of disease intensity in bacterial strains (Killiny and Almeida 2011). The interaction of bacteria with other organisms is greatly diversified from the biofilm formation to mutual interaction and up to pathogenic associations. The formation

and synthesis of certain specific proteins play an important role in such plant pathogen interactions. Among them certain proteins are toxic and enter within host cells and modify their physiology and colonization for the production of effector proteins leading to disease development (Tseng et al. 2009). The allelic difference in effector molecules in all pathogens is also an important factor in host specificity (Alfano and Collmer 2004). The genetic engineering strategies are not so easy due to plant defense response, inhibition of bacterial "vir" factors, and production of certain antimicrobial compounds for effective disease management required for the protection of host (Melchers and Stuiver 2000). A considerable amount of protection against various diseases has been accomplished, but the resistance against broad range remains to be explored (Rivero et al. 2012).

10.2.3 Environmental Effects

A prominent aspect of hosts and parasites basically depends on genetics of organisms, and hence it can be determined by coevolution. In laboratory conditions the evaluation of infection is determined by the environment in which hosts and parasites interact with other. The interaction of hosts and parasites is also regulated by environmental conditions for the strength and specificity. Additionally, the various constituents of interaction for host-parasite fitness are differentially manipulated by environment. In spite of all these conditions, the environmental variations are not frequently included in experiments studying the coevolution and theoretical models. However, most of the interactions of host-parasite occur in heterogeneous environments; hence it is important to include fluctuating environments during the theoretical and experimental studies of host-parasite coevolution (Wolinska and King 2009).

The traits of life history are not only dependent on specific genotype but also mediated by environmental conditions. The phenomena "reaction norm" result into versatile phenotypes from a single genotype according to environmental conditions (Stearns 1992). During extreme cases or non parallel slopes of reaction norm, if genotype A is better than genotype B under one set of environmental conditions and the case is opposite in another set of environmental conditions, the cross is known as genotype-by-environment interaction. Subsequently, the genotypic variation is conserved in population, if there is any possibility of adaptation under different environmental conditions throughout time and/or space (Byers 2005). Several studies have proposed continuously that the environmental conditions modify the strength of selection during host-parasite interactions and the host genotype experience less or more problem during infection according to environmental settings (Sandland and Minchella 2003). In 1960, the concept of "disease triangle" was suggested for consequence of disease in hosts (plants). Three factors basically involved in this concept for the regulation are the hosts, the parasites, and the environment. Before 20 years, the scientists of plant breeding group recognized another term "genotype-by-environment interactions" in the research field of host-parasite interactions. This term advocated that comparative resistance of cultivars to their

particular parasitic strains is governed by environmental conditions (Browder 1985). One of the best examples of this concept is in oat cultivar which gets fewer infections by rust fungus during the winter season, while high infection rate is found in summer season. However, this conventional process is entirely reversed during the infection of the same cultivar with another fungal strain.

The experimental studies are going on in the field of host-genotype-environment (H-G-E) interactions and parasite-genotype-environment (P-G-E) interactions under various conditions along with different genotypes of hosts and parasites (HG-PG-E). The statistical tests of these three-way interactions were conducted in barley growth and aphid reproduction. The various genotypes and presence of rhizobacterium (the rhizobacterium is taken as "environment" in this experiment) both affect the growth in barley and reproduction in aphid resulting in substantial HG-PG-E interactions (Tetard-Jones et al. 2007). The environment (rhizobacterium here) substantially changed the selection specificity in 31 out of 92 performed tests.

The nutrient availability and temperature are environmental factors tested most frequently during host-parasite interactions. Temperature greatly affects the physiological, biochemical, and behavioral processes in both hosts and parasites. In several studies, the temperature enhances development of parasites and exploitation of hosts and therefore disease occurrence (Thomas and Blanford 2003). The nutrient variability is able to alter specificity of hosts and parasites. For example, certain susceptible host genotypes can use the supplementary food during nutrient variability for their defense, while the rich nutrient availability might provide more suitable environment for parasite infection in other hosts (Laine 2007).

10.2.4 Epigenetics and Transgenerational Resistance

Plants can achieve immunity within their own lifetime, and pathogen interaction with plants also results in epigenetic modifications in cell that lead to immunization in the next generations. A study shows that the treatment of *Arabidopsis* with the β-amino-butyric acid (BABA), a SAR inducer component, and the avirulent strains of *P. syringae* pv. *tomato* inoculated on tomato plant led to enhanced disease resistance up to the next generation with faster and stronger expression of SA-dependent defense-related genes (Luna et al. 2012; Slaughter et al. 2012). Offspring from primed plants also shows enhanced resistance to the biotrophic oomycete *Hyaloperonospora arabidopsidis*. This phenotype persists in further one stress-free generation followed by increased resistance during pathogen attack in the next upcoming generations (Slaughter et al. 2012). Mutation in the npr1 gene can block the transgenerational resistance (Luna et al. 2012).

Transgenerational resistance of plants is regulated by certain epigenetic modifications such as DNA methylation and chromatin rearrangements. Control of transgenerational stress memory takes place through somatic homologous recombination of gene. *Arabidopsis* plants treated with flg22 or ultraviolet C show enhanced somatic homologous recombination in both parental and in the next four subsequent

generations (Molinier et al. 2006). Further studies focusing on the molecular mechanism of passing immunological memory to its subsequent generations are still in progress for better disease control mechanism in plants (Henry et al. 2013).

10.3 Conclusion

Plants have various cell surface receptors to discriminate the different PAMPs common in most of the microbes. Apart from isolating bacterial PAMP receptors, the research in host-parasite coevolution field is rapidly progressing toward the recognition of PAMPs and their respective receptors. Several PAMPs have been identified with their own receptor. An individual microorganism will efficiently be distinguished by several PAMP receptors. Many labs are specifying the enzymatic activities of effector molecules and recognizing their targets in host. The recognition of effector molecule targets will open the molecular action of PTI because the primary action of effector molecules is inhibition of PTI. Apart from this, the pathogen effector molecules may also contain virulence components which can be necessary for disease incidence. The battle between host-parasite interactions is a continuous process for their coevolution. In the coming years, there may be some new mechanisms revealed about host-parasite interactions. Explanation of mechanisms involved in controlling the coevolution of host-parasite interactions will be greatly affected by new techniques including rapid genome sequencing, gene editing, and development of computational methods along with bioinformatics to study the available genome information. Concurrent researches that make use of post-genomic technologies including system biology perspective will finally provide understanding of expression of all genes and proteins in hosts and parasites that are expressed during the battle of resistance simultaneously. These technologies will be very helpful to explore the complicated interactions between various pathways expressed during coevolution of hosts and parasites. Finally, the complete perception of molecular basis of host-parasite interactions will permit execution of these explored researches to make the hosts more resistant by adding novel combination of genes that are durable and identify various range of parasites.

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Host-Parasite Interaction During Development of Major Seed-Transmitted Viral Diseases

11

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Abstract

Being the most important mode of propagation of plants, disease-free seed is a basic requirement of progressive agriculture. However, many plant pathogens such as bacteria, fungi, virus, virus-like agents, nematodes, etc. are transmitted through seeds to the next generation. Seed transmission enables the earliest possible infection, which is a determining factor of disease severity. Plant viruses are the most important plant pathogens causing up to 96% yield losses. Although, seed transmissibility is presently known to occur for around 18% of plant viruses only, it is estimated that one-third of the plant viruses will eventually prove to be seed transmitted in at least one host. Nevertheless, the most likely mechanism of plant viruses' persistence between crop seasons is through the seeds, and even a seed transmission rate as low as 0.001 has the potential to initiate an epidemic. Therefore, study of the plant-pathogen interactions in seed-transmitted viruses is of utmost importance to help formulate preventive measures against such pathogens. Although the specific molecular mechanism of transmission of plant viruses through seeds is not completely understood till date, broadly, the mode of infection of the embryos (the future seeds) is known to be either direct (via mother plant) or indirect (via infected pollen); these mechanisms, however, may not be mutually exclusive. The modes of viral movement from infected maternal and paternal tissues to the embryo, the genetics of host-virus interaction, etc. are needed to be worked out for designing successful management strategies against seed-transmitted viruses.

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11.1 Introduction

Population outburst is a major issue in the present scenario of the world along with which comes the shortage of food production as an added crisis. Lots of efforts are going on for overcoming this alarming problem by agricultural scientists around the globe. As about 90% of food crops are grown through seed (Maude 1996), there has been an increasing importance on healthy seeds to boost agricultural production. Overall, seeds must be healthy and genetically pure and also have a high germination rate for better productivity. Seeds are highly prone to pathogen attacks since their development on parent plants till their germination because they are the principal sink tissues. Many plant pathogens like bacteria, fungi, virus, viruslike agents, and nematodes get transmitted through seeds to the next generation.

Although the plant pathogens are transmitted directly or indirectly through numerous agents, the seed transmission of pathogens has its own significance. Smith and Hewitt (1938) and McCubbin (1954) had enlisted some of the reasons why seed transmission is important. They are:

(a) The viability period of seeds is generally much longer than other planting materials.
(b) In association with seeds, pathogens survive for a longer period than they do in soil or separately in the environment. Longer virus survivability in association with seeds had been reported long before; for example, in case of *Prunus necrotic ringspot virus* in *Prunus pensylvanica* (Fulton 1964) and *Squash mosaic virus* in muskmelon (Rader et al. 1947).
(c) The host-parasite interaction in seed provides maximum opportunity for the next progeny infection.
(d) For establishment of a pathogen in a new locality, seed transmission is more successful than any other method.
(e) Seed transmission can facilitate the long spread of pathogens over transmission through vegetative propagules.
(f) If the pathogen-carrying seeds are planted in the field, pathogens get numerous infection points, and so it can spread more.

In 1699, apparently, Hellwig, for the first time, demonstrated the seed transmissibility of a plant disease with *Claviceps purpurea* of rye. Till the middle of the eighteenth century, many plant diseases in relation to the role of seeds in their dispersal were extensively studied.

As far as plant viruses are concerned, they are the most important biotic agents causing the highest yield loss. It is estimated that one-third of the plant viruses will eventually prove to be seed transmitted in at least one host. According to Stace-Smith and Hamilton (1988), approximately 18% of reported plant viruses are seed transmitted. The most common type of seed transmission of the viruses is found within the tissues of the embryo. However, transmission via seeds is relatively less due to the inability of most viruses to infect mother cells of infected plants together

with the inability of viruses to infect the developing embryo because of the general lack of plasmodesmatal connection with the endosperm.

The first seed transmission of a plant virus was suspected by Westerdijk (1910) and later by Allard (1914) in *Tobacco mosaic virus* (TMV) through tomato seed. Soon, another report about seed transmission of *Soybean mosaic virus* came up in the annual report of Connecticut Agricultural Experiment Station (Clinton 1916). Subsequently, several reports came on seed transmission of plant viral agents, such as *Lima bean mosaic virus* (McClintock 1917) and *Bean common mosaic virus* (BCMV; Stewart and Reddick 1917; Reddick and Stewart 1918, 1919). Today, crops of leguminous species have been found more vulnerable to seed-transmitted infection as there are reports of transmission of nearly 68 viruses through seeds in legumes. However, this assumption often attracts controversies, as many opponents claim that legumes are not more vulnerable to viral diseases as transmissibility is not a property of a virus or a host; rather it is an interaction of both the partners. Several researchers published a great deal of information on seed-transmitted viruses in terms of identification, ecology, epidemiology, and management aspects. So far, seed transmissibility has been reported in case of more than 231 viruses and viroids in different cultivated and weed hosts.

11.2 Seed Transmissibility and Associated Significance

With seeds being the means of sexual propagation, it is necessary to ensure that the seeds are disease-free. However, in case of certain viruses, which do not have multiple numbers of hosts, and those which cannot survive through seasonal variations, seed is the primary source of inoculum. Seeds are preserved for consumption and exchanged as germplasm among countries for various purposes (Chalam and Khetrapal 2008). These facts make seeds more valuable in the study of seed-transmitted diseases. Seed transmission not only facilitates the virus inoculum but also helps for long-period perpetuation of the infecting virus. Long-period perpetuation of BCMV in French bean seed and *Prunus necrotic ringspot virus* (PNRSV) in *Prunus pensylvanica* seeds are good examples, as these viruses can persist for 38 and 6 years, respectively (Walters 1962; Fulton 1964). Seed transmission is the most likely mechanism of viruses' persistence between crop seasons, and even a seed transmission rate as low as 0.001 can initiate an epidemic (Ryder 1973).

Some seed-transmitted viral diseases are cosmopolitan in their distribution. They greatly influence social facets of life by reducing the yield as well as the quality of plant products. The importance of seed-transmitted viral diseases and host virus interaction has been studied extensively. Hull (2004) described that seed transmission occurs for about one-seventh of the known viruses in one or more hosts. Two examples are generally cited to show the significance of seed-transmitted viral diseases: barley and lettuce had faced enormous yield losses due to *Barley stripe mosaic virus* (BSMV) and *Lettuce mosaic virus* (LMV), respectively, in the USA in the 1980s (Grogan 1980; Carroll 1983). According to an estimate, even 0.1%

incidence of LMV seed could result in a 100% crop loss (Broadbent et al. 1951; Grogan et al. 1952; Zink et al. 1956).

Besides, the effects of seed-transmitted viruses often come as an influence on seed viability. It had been shown that a mild strain of seed-transmitted hop infecting virus (PNRSV) caused a 20% reduction in germination, while a severe strain of the same virus could reduce the germination percentage by as high as 90% (Blattny and Osvald 1954). Similarly, seeds of spurrey infected by *Tomato black ring virus* (TBRV) were observed to germinate slower than healthy seeds (Lister and Murant 1967).

11.3 Economic Loss Due to Seed-Transmitted Viruses

Once successful virus infection occurs in plants, it lasts for lifelong and affects all the related growth parameters and yield of plant. The virus infection in crop causes yield loss, passage of virus from one crop season to another, and spread of disease to the neighboring crops. The loss of crop can be sustainable for annual crops; but in perennial crop like fruits, it imparts a major impact.

In the seed-transmitted viruses, among the earliest reports, a yield loss of 50–68% in bean was reported to occur due to occurrence of BCMV (Lockhart and Fischer 1974; Hampton 1975). More recently, from Western Australia, Coutts et al. (2008) have reported a 96% yield loss in lentil seed due to *Pea seed-borne mosaic virus* (PSbMV). The extent of crop loss due to viruses depends mostly on the disease intensity and distribution; however, it is often difficult to assess the exact loss caused by the seed-transmitted (and other) viruses. Multiple factors work against having an accurate yield loss data as:

- Variations in losses by a virus in a particular crop from year to year.
- Different degrees of loss among regions even within the same year.
- Differences in loss assessment methodologies, dependence on agronomical practices, etc.

Generally, yield losses are relatively greater if the plants are infected at early stages of development. Again, a mixed infection often leads to greater yield loss compared to an infection with a single virus. Although there are several reports available on major yield loss due to seed-transmitted viral disease, it is very difficult to get data on exact yield losses in different hosts and virus infections. Some of the important seed-transmitted viruses along with the percentage yield loss in host crop(s) due to their occurrence are listed in Table 11.1.

11.4 Mechanism of Seed Transmission

The specific mechanism of transmission of plant viruses through seeds is not well-understood. Many such viruses are transmitted through pollen. Pollen grains from infected plants may carry the virus either on outer exine or on inner intine. During

Table 11.1 Some important seed-transmitted viruses and the percentage yield loss by them in host crop(s)

Virus	Crop	% yield loss	Reference
Bean common mosaic virus	French bean	35–98	Shukla et al. (1994)
	Mung bean	31–75	Kaiser and Mossahebi (1974)
Bean yellow mosaic virus	Broad bean	≤59	Shukla et al. (1994)
Broad bean stain virus	Lentil	14–61	Makkouk and Kumari (1990)
Cucumber mosaic virus	Lupin	25–42	Bwye et al. (1994)
Lettuce mosaic virus	Lettuce	≤30	Shukla et al. (1994)
Pea seed-borne mosaic virus	Pea	11–36	Khetrapal and Maury (1987)
Peanut mottle virus	Groundnut	20–72	Shukla et al. (1994)
Peanut stripe virus	Groundnut	6–79	Shukla et al. (1994)
Soybean mosaic virus	Soybean	48–99	Tu (1989)
Tomato mosaic virus	Tomato	5–5	Walkey (1991)
Zucchini yellow mosaic virus	Cucurbit	0–99	Shukla et al. (1994)

fertilization, if the infected gamete unites with the egg cell, the resulting embryo may be infected. Union of the other gamete with polar nuclei may produce infected endosperm.

Cytoplasmic connections (plasmodesmata) of the mother plant with flower and developing seeds influence seed-transmitted infection; more cytoplasmic connection leads to higher infection. Indeed, viral transmission in legumes is more frequent due to presence of a greater number of plasmodesmata (Sastry 2013).

Ovule infection also occurs with several viruses which may move into different parts of the seed including the embryo sac. Viruses can enter the embryo sac from the nucellus till cytoplasmic connections exist. As soon as the membrane of the egg cell is formed, the movement of the virus ceases. The capability of a virus to successfully infect the ovule or pollen systematically leads to seed transmission.

In general, the embryo (the future seed) can get infected by two routes: directly from the mother plant or by pollens (reviewed by Johansen et al. 1994; Maule and Wang 1996). Because symplastic connection is cut off by meiosis, the direct route, however, is a problem. The process of embryo infection by either route is complex and involves multiple factors (Maule 2000). For embryo infection, the virus has to reach the floral meristem, which is generally not reached by long-distance movement through the phloem. However, though not experimentally proved, the *Cucumber mosaic virus* (CMV) was suspected to be transmitted through infected gametes at fertilization (Ali and Kobayashi 2010). Alternatively, the virus has to infect the embryo itself.

Wang and Maule (1997) studied the accumulation of *Pea early browning virus* (PEBV) and PSbMV in pea embryos using immunological techniques and distinguished these two routes of infection. Their findings are discussed below.

Some viruses like PEBV, nepoviruses, and cryptic viruses infect floral meristems and finally the gametes; however, not much has been understood yet about this mechanism. Direct embryo infection has been examined in PSbMV in detail (Wang

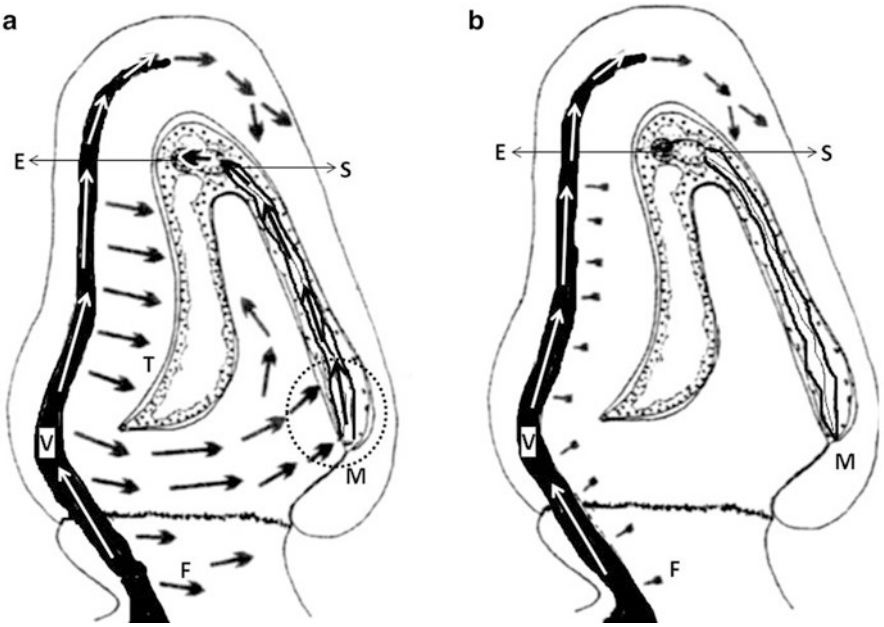

Fig. 11.1 The pathway to seed transmission of PSbMV in pea. (**a**) Systematic analyses of the immature seeds of different ages have identified the routes (arrows) of virus invasion in the pea variety, Vedette. The most consistent observation is that the virus must reach the micropylar area of the testa for seed transmission to occur, a location providing the closest point of contact between the testa tissues and the embryonic suspensor (encircled with dotted line). (**b**) In nonpermissive interaction the virus with the pea variety, Progretta, the virus' ability to spread into and/or replicate in the nonvascular testa tissues appears to be blocked (blockheads). *E* embryo proper, *F* funiculus, *M* micropylar region, *S* suspensor, *T* testa, *V* vascular bundle. (Adapted from Hull 2004)

and Maule 1994). It was hypothesized that for embryonic infection, the virus has to move through the testa of the immature seed and must reach the micropylar region of the seed which is in close contact with the suspensor. Much later, Roberts et al. (2003) had worked out the model of PSbMV transmission (Fig. 11.1). They found that first, the virus infects the testa and then invades the endosperm through the plasmodesmal channels. During the early development of seeds, PSbMV invades the suspensor cells through transient vesicles. These vesicles are present in the micropylar region where the suspensor is anchored to the endosperm; therefore, during this period, the micropylar region must carry the virus. Subsequently, the virus reaches the embryo through the pore-like structures which are located in the sheath that separates the embryo from the suspensor. However, as the suspensor degrades in the later stages, there exists only a short window for the virus to use this route of embryo infection. A similar mechanism of transmission of *Soybean mosaic virus* (SMV) has also been proposed (Fig. 11.2). The model explains that SMV first invades the ovule wall; the virus reaches the micropylar region and later between the testa and suspensor cells in later developmental stages of the embryo. The virus

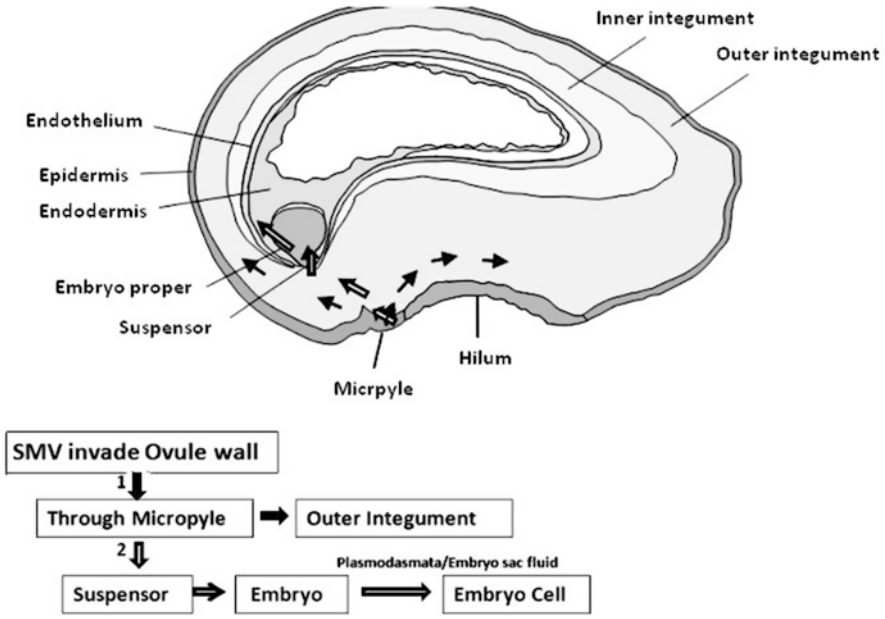

Fig. 11.2 Proposed model of embryo invasion by SMV. SMV invades the embryo through the ovule wall, the micropyle region, and, subsequently, the testa-suspensor interface. The virus replicates in the embryo cells and disperses through plasmodesmata or in embryo sac fluid. (Adapted from Bashar 2015)

enters in the embryo before degeneration of the suspensor. Inside the infected embryo, the virus moves either by plasmodesmata or embryo sac fluid (Bashar 2015).

Examples of pollen-transmitted viruses include *Alfalfa mosaic virus* (AMV; Frosheiser 1974), *Turnip yellow mosaic virus* (TYMV; de Assis and Sherwood 2000), etc. In pollen transmission, two mechanisms appear to operate: infection of the embryo and infection of the mother plant. For some viruses, such as BSMV (Carroll 1974), *Tobacco ring spot virus* (TRSV; Yang and Hamilton 1974), PNRSV (Aparicio et al. 1999), AMV (Pesic et al. 1988), etc., the egg cell is infected presumably via the infected sperm (or the sperm nucleus). On the other hand, viruses such as TMV (Hamilton et al. 1977) were found in the exine of mature pollens from infected plants; this indicates that there is a second mechanism for pollen transmission. The virus could be picked up by growing pollen tubes and actually transmit the virus by mechanical means to the ovule.

In case of some viruses, both direct and indirect invasion may take place together, for example, in BSMV infection in barley (Mandahar 1981). However, in direct embryo invasion, how the viruses can cross the boundary between the parent and progeny is still not understood.

11.4.1 Direct Invasion

The embryo of developing seed may get infected by inoculation of viruses after flowering. In this mode of invasion, the embryo colonization occurs after fertilization by sequential virus movement into the seed, from the micropylar region of the maternal testa to the endosperm, the suspensor, and finally the embryo. Wang and Maule (1992, 1993) proved this type of invasion in pea. In developing seeds of pea, the movement of PSbMV was observed by using serological techniques. The virus was generally found in the funiculus before fertilization, but rarely in unfertilized ovules. Following fertilization, the virus was detected in the immature and mature seeds including the developing testa, the endosperm, and the suspensor.

One major conceptual problem existed in the direct invasion of the embryos by seed-transmitted viruses. It was not clear how the virus moves from maternal to embryonic cells, because the symplastic connections are severed between maternal and embryonic cells early during meiosis. Passage of only small nutrient molecules was believed to be allowed by this barrier through apoplastic transport (Tegeder et al. 1999, 2000). Therefore, Roberts et al. (2003) had investigated by electron microscopy of PSbMV models to understand how the virus moves from testa to the endosperm and from endosperm to suspensor cells. Although proper plasmodesmata were not observed, the results showed an occasional distortion of the cell wall giving a passage to virus by means of a reminiscent of plasmodesmal cavities. However, further works were needed to decide whether these symplastic connections are constitutive or specifically induced by seed-transmitted viruses (Roberts et al. 2003). The same group had also described pore-like structures in the embryo sheath, at the base of the suspensor cells, and presumed that these structures could be instrumental in transfer of viruses between endosperm and suspensor cells.

It is not clear how viruses move into embryos, but the contact point of the testa with the suspensor might be the possible route. Although plasmodesmata are observed, the cell wall at the contact point is very convoluted. Therefore, Wang and Maule (1993) suggested that the virus could possibly move by an as-yet unidentified mechanism involving no plasmodesmata or that viruses could also induce new plasmodesmata for the movement.

However, Kohnen (1992) had experimentally shown that at least in case of PSbMV, both direct and indirect invasion routes for embryo infection occur.

11.4.2 Indirect Invasion

In the process of indirect invasion, the developing gametes get infected before fertilization; either or both male (pollen/pollen mother cell) and female gamete (ovule/megaspore mother cell) may get infected (Carroll 1981). It can be speculated that the entry of viruses into tetrad and microspore is possible till the formation of pollen grains, i.e., before maturation of the pollen. Hence, the uniformity of microspore maturation may also affect the percentage of seed transmission. *Tobacco rattle tobravirus* particles were found in premeiotic pollen mother cells and, later, in

pollen (Gaspar et al. 1984) of soybean. The high seed transmissibility of TRSV in soybean was seemingly related to the capacity of TRSV to invade meristematic tissue and to infect the megaspore mother cells. Another pollen-transmitted virus is AMV (Wilcoxson et al. 1975) in alfalfa. Indirect invasion of the embryo has also been shown in cryptic viruses (Kassanis et al. 1978), some other tobraviruses (Wang et al. 1997), and nepoviruses (Hull 2004). This property is attributed to the ability of those viruses to invade the meristematic cells (Maule and Wang 1996). It was proposed that the ability of certain viruses to infect gametes does not depend on specific mechanisms of viral transports into the meristem; rather this ability depends on circumvention of post-transcriptional gene silencing (PTGS). However, the same mechanism may not work in case of all seed-transmissible viruses. It had been shown in TMV infection in *Arabidopsis* that meristem invasion does not depend on PTGS (Schwach et al. 2005).

Extensive studies have been conducted on seed transmission of BSMV in barley (Carroll 1972; Mayhew and Carroll 1974; Carroll and Mayhew 1976a). They studied the cytological changes in the floral meristems during meiosis and embryo formation in relation to the distribution of a seed-transmitted strain (MI-l) and a non-seed-transmitted strain (NSP) in the reproductive tissues. They found that seed transmission of BSMV was determined by the ability of BSMV to invade male and female reproductive meristems very early in their development, thereby infecting the embryo indirectly (Carroll 1981).

11.4.3 Cell-to-Cell Movement

Unlike animal viruses, where cell receptors facilitate the uptake of the infecting virus into the cell, and thus allow the virus to exploit extracellular environment for dissemination, plant viruses remain restricted to intracellular compartment or symplast. Their movement from cell to cell occurs through highly regulated symplastic channels, the plasmodesmata, mediated by viral movement proteins (MPs). MPs alter the gating capacity of plasmodesmata and allow infectious viral genomes to pass to the neighboring cells. Gradually, the viral particles go to the vasculature and systemically move through the phloem sieves. Therefore, viruses can move to the cells requiring photo-assimilates. Viruses, however, differ in their ability to pass from developed vasculature to incompletely differentiated or meristematic tissues. For example, while nepoviruses invade shoot apex extensively, *Maize streak viruses* appear to be limited to vascular bundle.

11.4.4 Host and Viral Determinants of Seed Transmission

Though not clear, it is believed that the two mechanisms of seed transmission, viz., infection of gametes and direct invasion, are controlled by different set of host genes, and they must act in one or more than one stage of infection. Most of the works on determinants involved in seed transmission have been done in PSbMV

and BSMV. In PSbMV infection of pea, using crossing techniques, it was found that resistance determinants against seed transmission are indeed nuclear genes and that this is a quantitative trait with a few maternal genes having incomplete dominance (Wang and Maule 1994). However, in case of BSMV infection in *Hordeum vulgare*, a single recessive host gene was presumed to be involved in such a host-virus interaction (Carroll et al. 1979). It could be generally predicated that genes that regulate the ability of a virus to move locally or long distances might affect the invasion of the gametes or the embryo. At the same time, an alteration of the replication capacity of a virus might affect the virus titer needed for such invasion. These factors could also be involved in spread of infection through vegetative tissues. Therefore, separating specific factors for seed transmission may be very difficult. It is also possible that for a given isolate, different genetic determinants may work at different stages of infection process.

Meanwhile, considering the fact that isolates of the same virus differ in transmissibility, generalization of the viral genes involved in seed transmission is also difficult. Due to this difficulty, experiments on the viral determinants have been conducted by reassortment or pseudo-recombination of divided genome segments or by molecular techniques such as sequence comparison and mutagenesis or the formation of chimeric viruses. It has been found that the seed transmissibility of viruses is determined by different genome components and genes in different viruses. Pseudo-recombination experiments with the two RNAs of some nepoviruses showed that seed transmissibility was markedly dependent on the viral genome component called RNA1. The genome component called RNA2 had an additional smaller influence (Hanada and Harrison 1977). Similarly, in PEBV, RNA1 was found to be the major determinant, with RNA2 playing a minor role in seed transmissibility (Wang et al. 1997). Removal of the 12 kDa gene from RNA1 almost completely abolished seed transmission. The deletion mutant also accumulated poorly in anthers and carpels, as well as in pollen grains or ovules; this suggests that this gene is involved in the infection of the gametic cells (Wang et al. 1997). In BSMV, pseudo-recombinants between a seed-transmitted and a non-seed-transmitted strain showed that for seed transmission of the virus, RNAγ and RNAβ play predominant roles (Edwards 1995). The determinants of seed transmission in RNAγ are the 5′-untranslated leader and a 369 nt repeat in the γa and γb genes. It was also shown that a complex interaction between the RNAγ leader, the γb gene, and RNAs α and β is pivotal for the seed transmissibility of the virus. Because RNAβ of BSMV is known to code for the MP and RNAγ codes for polymerase, these results indicated involvement of viral replication and movement in seed transmission of BSMV. PSbMV was also studied making recombinants of a highly seed-transmitted and poorly seed-transmitted isolate. It was found that the 5′-untranslated region, the helper component protease (HC-Pro), and the coat protein regions are involved in its seed transmission, with the HC-Pro having the major role (Johansen et al. 1996). These regions of PSbMV are also important for viral replication and movement. Similarly, in PEBV, the 12 K was presumed to be important for seed transmission (Wang et al. 1997). Pseudo-recombination studies with strains having different seed transmissibility showed that this ability of the virus was associated

with RNA1 of raspberry ringspot and tomato black ring nepoviruses (Hanada and Harrison 1977) and to RNA1 of *Cucumber mosaic virus* (*Cucumovirus*) (Hampton and Francki 1992). However, till today, functional links of these proteins with their role in seed transmission have not been established.

Recently, it was shown that seed transmission of SMV is influenced by P1, HC-Pro, and capsid proteins (Jossey et al. 2013).

11.5 Host Resistance to Seed Transmission

A very interesting feature of virus-host relationship in seed-transmitted disease is that embryos are generally protected against viruses invading the mother plants. This may result from various physical as well as physiological/biochemical barriers to virus entry and replication in reproductive tissues.

Within a rapidly growing meristematic cell, the inner environment must be quite distinct from a differentiated cell. This difference could certainly limit the viral entry into developing seeds, and this could be either a general host mechanism or a virus/host-specific adaptation. Proof of a general physiological barrier was provided by Citovsky et al. (1993) in TMV-P30 MP. It was postulated that the ability of TMV to invade plant tissues may be influenced by phosphorylation of P30 by a cell wall-associated kinase; the kinase was found to be expressed in a spatial and temporal manner. Meanwhile, in several crops, including barley (against BSMV), pea (against PSbMV), alfalfa (against AMV), and soybean (against SMV), variety-specific resistance against seed-transmitted infection had been reported. While the resistance in barley was found to be regulated by a single recessive gene (Carroll et al. 1979), resistance in pea was controlled by multiple genes (Wang and Maule 1993). The genetics of resistance in soybean and alfalfa have not been worked out. Below, we discuss some suggested mechanisms of differential seed transmissibility of viruses.

11.5.1 Movement and Replication

Restriction of virus movement or replication has not been established as a mode of resistance against seed-transmitted viruses. However, there are certain differential rates of seed transmission implicated with this mechanism. Walter et al. (1992) had suggested that seed transmission of *Tobacco streak virus* (TSV) in *Phaseolus* was influenced by restriction of viral movement. Because viral invasion and establishment are essential for seed transmission (Hanada and Harrison 1977), it was speculated that along with floral and gametophytic tissue, the virus could possibly not invade the embryo meristem too.

Similarly, restricted viral replication could also influence infection and accumulation in floral tissues. This could work both directly, by limited replication in meristems, or indirectly, by incomplete systemic movement. Hampton and Francki (1992) assumed in case of infection of CMV in *Phaseolus vulgaris* that seed transmission is possibly influenced by replication of the virus in the embryo. Meristematic

cells might contain viral inhibitors, and they might lack host factors needed for virus replication. The efficiency of seed transmission had been shown to depend on presence of BSMV in barley (Carroll and Mayhew 1976a, b) and PSbMV in pea (Kohnen 1992) in their reproductive tissues.

11.5.2 Inactivation

Seed transmission is not only determined by viral invasion of the embryo but also by survival of virus during seed formation, storage, and germination. Seed transmission could be limited by virus inactivation in the embryo tissues during maturation. Increased virus inactivation with maturity has been reported in case of AMV in alfalfa (Bailiss and Offei 1990) and SMV in soybean, and *Southern bean mosaic virus* (SBMV) in bean (Bowers and Goodman 1979) inactivation may progress even during storage (Powell and Schlegel 1970; Mayee 1977). However, some other seed-transmitted viruses are stable in the embryo and remain infective till the seed remains viable (Lister and Murant 1967; Tomlinson and Carter 1970; Laviolette and Athow 1971; Vorra-Urai and Cokbain 1977).

Mechanism of virus inactivation in seeds is unclear, but this inactivation is likely associated with physiological changes during maturation such as nutrient relocation, cessation of cellular processes, and increase in the concentrations of inhibitors such as phenols and quinones together with dehydration.

11.5.3 Inhibition of Replication at Germination

Some plant viruses are not seed transmissible due to their inhibited replication during seed germination. No studies have been done to show that the cellular components that are synthesized during seed germination affect viral replication initiation during seedling emergence. The presence of inhibitors of viruses in seeds is known from in vitro studies; therefore, interpretation of these studies is difficult (Hanada and Harrison 1977; Uyemoto and Grogan 1977; Hajj and Stevens 1979). Nemeth and Kobler (1982) reported that the incidence of *Plum pox potyvirus* (PPV) that could be detected by ELISA in apricot embryo axes correlated to the rate of seed transmission; however, PPV was detected at a much higher rate in cotyledons. Therefore, an unknown mechanism of virus inhibition during germination of apricot seeds was suggested. Such confusions, which were common during initial applications of ELISA, were probably due to common occurrence of viral coat protein in seeds from infected plants.

11.6 Factors Influencing Seed Transmission

11.6.1 Virus Strain/Isolate

Seed transmissibility of viruses varies not only among different viruses but also among strains/isolates of the same virus. Two isolates of PSbMV (P-1 and P-4) were seed transmitted at a rate of 24% and 0.3%, respectively, in *Pisum sativum* (Johansen et al. 1996). Similarly, four isolates of PMV showed different frequencies of seed transmission in Starr peanut (Adams and Kuhn 1977).

11.6.2 Mixed Infections

The rate of seed transmission of viruses varies depending on single or multiple infections of the same host. However, the mechanism of this change in the rate of seed transmission in mixed infections is not well-understood. One of the earliest reports of increased seed transmissibility in mixed infection was that of SMV in soybean with *Bean pod mottle virus*; while SMV alone was transmitted only at a rate of 6%, a mixed infection of these two viruses leads to 11% increase of SMV (Ross 1963). Similarly, although the frequency of seed transmission of *Southern bean mosaic virus* (SBMV) was 12% in cowpea, it increased to 20% in the presence of *Cowpea chlorotic mottle virus* (CCMV) (Kuhn and Dawson 1973). Seed transmissibility of *Turnip yellow mosaic virus* (TYMV) in *Arabidopsis thaliana* also increased to 70% from 31% when the host is doubly infected with TYMV as well as TMV (de Assis Filho and Sherwood 2000).

11.6.3 Host Species

Seed transmission of a virus also depends on the host species; that was noticed as early as 1935 by Hewitt. Seed transmission of PMV in peanut, but not in cowpea or soybean, was reported by Bock and Kuhn (1975). Later, voluminous amounts of work have been done to examine the dependence of seed transmissibility of the plant host species; and considerable amount of differences has been reported. For example, LMV was very commonly transmitted in seed of some but not all lettuce varieties (Couch 1955). This kind of difference is presumed to be mostly related to the susceptibility or resistance of a host to a specific virus/strain.

11.6.4 Stage of Infection

Infection prior to flowering results in embryonic infection that may lead to maximum seed transmission (Schippers 1963). For example, the seed transmission of BCMV in a bean cultivar (Similac) was 40%, 9%, and nil, and in another cultivar (Pubbele Witte), the transmissibility was 41.8%, 2.8%, and 10.1%, when the host

plants were inoculated at 10, 20, and 30 days after sowing, respectively (Morales and Castano 1987).

Highly determinant plants that flower over a short period might give no seed transmission from inoculations after first flowering (Eslick and Afanasiev 1955). But, if infection happens early enough, indeterminate plants like peanut may produce seeds that can transmit virus. However, if plants are infected after flowering, there is no infected seed produced, in general (Bos 1977). Early infection leading to high seed transmissibility has been shown in several host-virus combinations. Chitra et al. (1999) have shown that inoculation of both ToMV and TMV at any growth stage leads to entry of the viruses to seeds of tomato and bell pepper. Though inoculation at flowering and also during fruit set stage can lead to virus entry into seeds, the viral concentration in seeds is generally higher, if infection occurs at an early growth stage.

11.6.5 Production of Disease Symptoms

Seed-transmitted viruses generally induce mild symptoms; however, the symptoms caused by some virus-host combinations do limit the seed transmission. In infection of some viruses, flower development is highly affected that results in decreased seed set and reduced potential of seed transmission. An example of such differential seed transmission was shown in TSV. While early infection with TSV-A strain resulted in seed transmission, early inoculation with TSV-W strain limited flower production, seed development, and seed transmission (Ghanekar and Schwenk 1974).

Pollen and ovule fertility and their competitiveness are negatively influenced by virus infection. *Tomato aspermy cucumovirus* infection interferes with meiosis, producing sterile pollens and ovules (Caldwell 1952). Pollen quality is also often affected by infection of virus. Yang and Hamilton (1974) found that TRSV-infected soybean plants produced less pollen, which too germinated poorly and pollen tubes grew more slowly.

High seed abortion rate was observed in birch infected with CLRV (Cooper et al. 1984) in *Chenopodium* infected with *Spinach latent ilarvirus* (SpLV) or in *Nicotiana* infected with *Arabis mosaic nepovirus* (ArMV; Walkey et al. 1985). Virus-infected plants give rise to smaller-sized seeds with reduced germination rate that produced smaller seedlings with a competitive disadvantage (Hemmati and McLean 1977; Suteri 1981; Cooper et al. 1984; Hicks et al. 1986; Sdoodee and Teakle 1988).

11.6.6 Environmental Factors

Some environmental factors influence seed transmission of viruses probably by affecting the plants' physiology by manipulating the balance between virus replication and the plant growth or by affecting the virus stability. Temperature is a major environmental factor that affects seed transmission of viruses. In alfalfa, seed transmission rate was more at 29 °C compared to 18 or 24 °C (Frosheiser 1974). Hanada

and Harrison (1977) found that the rate of transmission of different strains of the same viruses is also influenced by temperature. Similarly, it was reported that the optimal temperature for seed transmission of SMV was 20 °C, while for virus accumulation was 25 °C (Tu 1992). However, storage temperature does not seem to affect the seed transmission rate, at least in case of AMV in alfalfa, where the seed transmission rate did not change even after storage for 5 years at various temperatures (21–27 °C, 4 °C, and − 18 °C; Frosheiser 1974).

11.7 Viral Factors of Seed Transmissibility

Viral gene products plausibly associated with seed transmission have not been worked out, because seed transmissibility is not a definitive characteristic of any known virus group and also no obvious cellular analogs are known to exist. Meanwhile, sequence of related seed-transmitted and non-seed-transmitted virus isolates has not depicted any specific domain, possibly associated with this phenotype.

11.7.1 Pseudo-Recombinant Approaches

Pseudo-recombinants of viruses are hybrid viruses with different genomic components from more than one related viruses. Use of pseudo-recombinants for the first time demonstrated that the seed transmission phenotype was associated with RNA1 of *Raspberry ringspot virus* and TBRV (Hanada and Harrison 1977).

In CMV too, the seed transmissibility was correlated with RNA1 by pseudo-recombination studies (Hampton and Francki 1992). In these studies, however, the rate of seed transmission of pseudo-recombinants was lower than parental strains. This showed that some incompatibility among heterologous RNAs led to alteration of seed transmission (Hampton and Francki 1992). In all the above viruses, however, RNA1 encodes proteins associated with replication suggesting a possible relationship between viral replication and seed transmission.

11.7.2 Chimeric Virus Approaches

Better understanding of the viral factors determining seed transmission was achieved by using full-length, infectious clones of highly seed-transmitted and poorly seed-transmitted plant viruses. In BSMV, ND18 is efficiently seed transmitted, and CV17 is a poorly seed-transmitted virus. Infectious RNA was generated from cDNAs and then pseudo-recombinants were generated. Using them, it was found that the seed transmissibility factor resides in RNAγ. More studies using chimeric RNAγ showed that three regions of this RNA – the 5′-noncoding region, the repeated region of the γa gene, and the γb gene – determine the seed transmissibility of BSMV (Timian

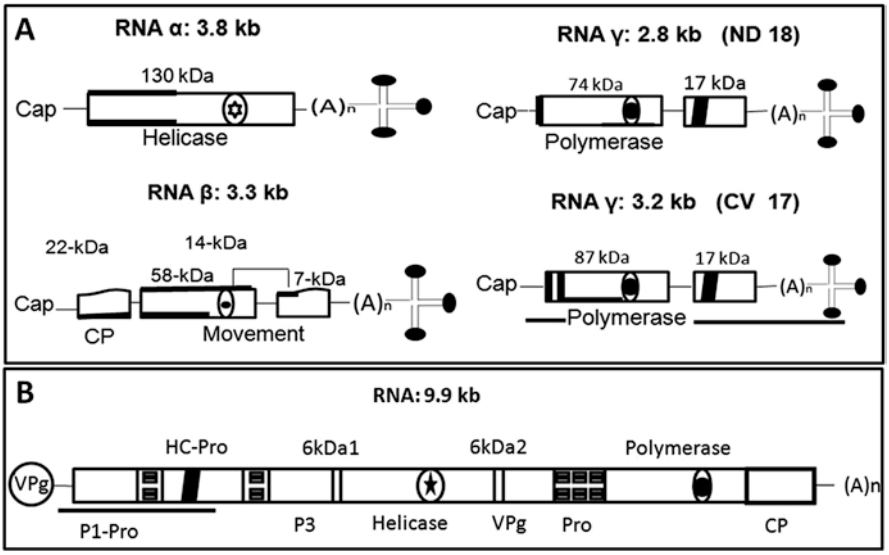

Fig. 11.3 (**a**) Schematic representation of genome structure and organization of *Barley stripe mosaic virus* (*Hordeivirus*). Genome segments reported to include primary determinants of seed transmission are marked with a solid bar (**b**) Schematic representation of genomic structure and organization of *Pea seed-borne mosaic potyvirus*. Genome segments reported to include primary determinants of seed transmission are marked with a solid bar. (Adopted from Johansen et al. (1994) (, protease; / , NTP-binding motif, characteristic of helicases; , RNA-dependent RNA-polymerase sequence motif; , cysteine-rich region; , 37 bp repeat of BSMV; Cap, 5′-cap structure; , tRNA-like structure; (A)n, Poly(A) sequence; HC-Pro, helper component proteinase; VPg, genome-linked virus-encoded protein; CP, capsid protein))

1974; Edwards et al. 1991) (Fig. 11.3a). Later, it was shown that the γb gene of BSMV actually works as a PTGS suppressor (Yelina et al. 2002).

Similar studies in PSbMV had showed that the greatest influence on seed transmissibility was given by the 5′-terminal fourth of its genome, where the P4 sequences reduced seed transmissibility rate from 35% to 3.5% (Johansen et al. 1993). The P4 sequence contains the 5′-untranslated leader, the N-terminal protein, the P1-protein, and two-thirds of the helper component protease (Fig. 11.3b). Some details of seed transmission factors of *Tobacco etch virus* have also been worked out (Carrington and Freed 1990).

The above findings provide evidence that replication and/or movement of the viruses may strongly influence their seed transmissibility. However, as the precise mechanisms of replication, movement, and their regulation in viruses are not well-known yet, the specific effects of these processes on seed transmission remain enigmatic.

11.8 Few Important Seed-Transmitted Viruses

A few seed-transmitted viruses have been found to be highly important and generally devastating during the recent history. Significance of these viruses in cereals, fruits, vegetables, and pulse crops has caught attention from several aspects, viz., virus perpetuation, perennation dissemination, ecological significance, as well as economic concern for plant growers. A few important seed-transmitted viruses have been mentioned in this section.

11.8.1 *Tomato Yellow Leaf Curl Virus* (TYLCV)

TYLCV is a *Begomovirus*, belonging to the family *Geminiviridae*, and is a major virus infecting tomato worldwide. Although begomoviruses were thought to be non-seed transmissible, recently, however, it was shown for the first time that *Sweet potato leaf curl virus* belonging to the genus *Begomovirus* does transmit through seeds in sweet potatoes (Kim et al. 2015). The same group (Kil et al.), again in 2016, reported about the seed transmissibility of TYLCV working with two strains of the virus.

The ssDNA genome of TYLCV codes for six open reading frames (ORFs): V1 and V2 in the virion sense orientation and C1, C2, C3, and C4 in the complementary orientation. *v1* and *v2* gene codes for the V1 and V2 protein which are basically the coat protein and pre-coat protein, respectively (Navot et al. 1991). Coat protein is known to encapsulate the ssDNA and form the virus particle to protect the viral DNA, whereas the pre-coat protein is thought to be involved in movement of the virus. The coat protein is also an essential component for effective insect transmission of TYLCV. The six open reading frames encoded by the viral genome are V1, V2, C1, C2, C3, and C4. C1 protein is better known as the Rep protein and well-known for its involvement in viral replication. C2 protein is a pathogenicity determinant and is localized in the nucleus of the host plant cell. C3 protein enhances viral DNA accumulation approximately 50-fold (Glick et al. 2009). C4 is considered as an important symptom determinant (Krake et al. 1998).

The seed infectivity was found to be 20–100%, and the average transmission rate to seedlings was also 84.62% and 80.77%, for the two strains of TYLCV. PCR analysis of leaf and seed samples from TYLCV-susceptible (cv. Seogwang) and TYLCV-tolerant tomato plants (cv Bacchus) agro-inoculated with TYLCV showed that TYLCV DNA accumulated more in case of the susceptible variety. This confirms that TYLCV transmits through seeds and seedlings (Kil et al. 2016). An investigation was done by the same team to check the reason for the tolerance and exclusive presence of *Ty-1* and *Ty-3a* genes and has been confirmed by PCR-RFLP analysis in case of the tolerant varieties.

11.8.2 *Barley Stripe Mosaic Virus* (BSMV) *(Hordeivirus)*

In temperate climates, barley (*Hordeum vulgare* L.) is grown as a major cereal grain that is used mainly as animal fodder and for breweries. The main hosts of BSMV, a *Hordeivirus*, are barley and wheat. There are several reports available on crop yield loss due to seed transmission of BSMV. In 1960 in the USA, in winter wheat, BSMV had caused yield reduction by 19% (Fitzgerald and Timian 1960). Another famous example of high yield loss in barley caused by BSMV in Montana, USA, was estimated to be more than US$ 30 million, during 1953–1970 (Carroll 1983). Later, Catherall (1972) reported that about 62% yield loss in barley cultivar Akka was caused by BSMV.

11.8.3 *Lettuce Mosaic Virus* (LMV)

Lettuce (*Lactuca sativa*) is an annual plant and most often grown as a leaf vegetable. North America is an important market for this crop due to its high demand. LMV is a potyvirus containing RNA genome with filamentous virus particle. LMV particles are long, flexuous rods with approximately 750 × 13 nm. The viral genome is composed of 1 molecule of a single-stranded, positive-sense RNA with 10,080 nucleotides (Revers et al. 1997). The viral genomic RNA has a viral-encoded protein and a poly-A tail covalently linked at the 5′-end and at the 3′-end, respectively, and contains a single ORF which encodes a large polyprotein with 3255 amino acids. This polyprotein undergoes self-cleavage resulting in eight to nine viral proteins (Shukla et al. 1994; Revers et al. 1997). Different isolates of LMV are classified on the basis of symptoms in various hosts (McLean and Kinsey 1962; Pink et al. 1992).

In lettuce crop seed transmission has been reported to occur through pollen but mostly maternally (Ryder 1964). In the USA, LMV had caused a great loss to the lettuce fields of California due to June yellows disease (Grogan 1980). Even a small amount of seed infection (0.1%) could result in total crop loss of lettuce (Broadbent et al. 1951; Grogan et al. 1952; Zink et al. 1956).

11.8.4 *Bean Common Mosaic Virus* (BCMV)

Beans are used as food and fodder worldwide. Bean is a common name for large seeds of several genera in the family Fabaceae/Leguminosae. The seed-transmitted virus, BCMV, infects bean crops and has been reported to cause major yield losses. BCMV is a potyvirus, comprises of ssRNA genome, and holds the position in family *Potyviridae*.

The seed transmission is rather common and probably the most important source of initial crop infection for BCMV in bean. Numbers of workers reported yield loss of 50–68% due to BCMV (Lockhart and Fischer 1974; Hampton 1975). According to other workers (Reddick and Stewart 1919; Crowley 1957; Schippers 1963), up to 83% seeds of diseased plants may be infected. There are reports available that if the

plant gets infection after flowering, it does not produce infected seeds (Nelson 1932; Schippers 1963) and that the distribution of infected seeds in pods is erratic (El-Attar et al. 1964). Quantz (1962) reported that the virus is found in the embryo and cotyledons but seldom in the seed coat.

11.8.5 *Broad Bean Stain Virus* (BBSV)

Like BCMV, BBSV is also an important seed-transmitted virus infecting the legume crops. BBSV is a *Comovirus* and belongs to the family *Secoviridae*. The seed transmission rates of *Broad bean stain virus* (BBSV) varied between 0.2% and 32% in 19 lentil genotypes (Makkouk and Kumari 1990; Kumari et al. 1996). Al-Khalaf et al. (2002) reported the variation in seed transmission of BBSV in lentil on the basis of genotype variability and seed size.

11.8.6 *Peanut Mottle Virus* (PMV) and *Peanut Stunt Virus* (PSV)

The peanut (*Arachis hypogaea*), commonly known as groundnut, is a widely grown legume crop in tropics and subtropics. Both the viruses, PMV and PSV, are economically important and belong to the genera *Potyvirus* and *Cucumovirus*, respectively. There are reports available of seed transmission for both PMV and PSV.

The reduction of yield due to *Peanut mottle virus* (PMV) from 31% to 47% was reported in Georgia (USA), and yield losses exceeded around $10 million per annum (Paguio and Kuhn 1974; Kuhn et al. 1978). In Virginia (USA), *Peanut stunt virus* (PSV) caused 80% loss of matured nuts in several commercial peanut fields (Culp and Troutman 1967).

11.9 Conclusion and Future Strategies

Seed transmission of plant viruses provides an ideal initiation point for the establishment of diseases in the field. Seed transmission enables the earliest possible infection, which is a determining factor of disease severity. Meanwhile, seed transmission provides a high number of initial inoculation sources. Management of viruses, especially the seed-transmitted viruses, for the above reasons, is therefore a challenge. Traditionally, virus management often involves management of the animate vectors by various means (as definite viricides are not yet available) as well as various cultural practices to remove virus inoculum. Quarantine has been a major and important strategy to prevent long-distance dispersal of viruses. However, the advanced methods of virus detection with serological and molecular means have revolutionized the virus detection strategies, which are now an integral part of the quarantine. Meanwhile, integrated management strategies have been worked out for many important viruses, and these have been found to be quite useful. Lately, there has been increasing attention on these integrated strategies too.

However, multiple details of the seed transmission of plant viruses are still unexplored and/or unknown. It could prove prudent in distinguishing the generally seed-transmitted viruses from the viruses dependent upon seed transmission for perpetuation, because the underlying mechanism of host-virus interaction in these two strategies could differ considerably. Research should possibly aim at identifying the host and viral genes/loci involved in host-virus interactions and the actual modes of these interactions. Regarding the mechanism of seed transmission of viruses, still certain basic questions, such as the mode of viral movement from infected maternal tissues to ovules or to the developing embryo, the route of virus movement from internally infected pollen grains to intracellular sites in the ovule, the mechanism of exclusion of viruses from the developing embryos, etc., have not yet been clarified. Therefore, the future works should take up these challenges. Besides, understanding of the ecology of known or potential important seed-transmitted viruses is a basic prerequisite for developing strategies for their management; and the study should focus on these areas as well.

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Part V

Diversity of Seed-Borne Phytopathogens

Diversity of Seed-Borne Fungal Phytopathogens

12

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Abstract

Contaminated seeds are the primary sources that are responsible for the spread of seed-borne diseases. Several seed-borne diseases are responsible for low productivity, but fungal seed-borne diseases are the most important. After harvesting, the storage and climate are the main determinants for the increase in microflora propagules on seed coats in different geographic areas. Crop production mostly depends on seed quality that comes in contact with different groups of fungal pathogens, responsible for disease distribution and reduced economy of agriculture worldwide. Stored seeds have huge amount of different microflora on economically important crops which are widely considered as pathogenic, phytotoxic, and carcinogenic. The aberrations like discoloration of seed, necrosis, delayed germination, dormancy, and other seed abnormalities happen on seed due to several seed-borne fungal pathogens. This chapter includes the detail description of distribution, biology, and symptoms of major seed-borne phytopathogens, viz., *Fusarium*, *Tilletia*, *Alternaria*, *Curvularia*, *Penicillium*, and *Aspergillus*. This chapter also includes the cultural resource available at Indian Type Culture Collection which will be useful in research associated with seed-borne fungal phytopathogens.

12.1 Introduction

Seeds are core germplasm which are responsible for distributing several plant pathogens for long distance. Earlier many authors had reported that contaminated seeds are the main sources for the spread of several plant diseases worldwide or

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Table 12.1 Important seed-borne cereal diseases incited by fungal pathogens

Crops	Seed-borne diseases
Rice	Blast, brown spot, false smut, pecky rice, root rots, foot rot, bakanae, seedling blight, and *Alternaria* leaf spot
Wheat	*Alternaria* leaf blight, sooty molds, kernel smudge, crown rot, seedling blight, dryland root rot, flag smut, Karnal bunt, loose smut, *Fusarium* head blight, spot blotch, and storage molds
Maize	Anthracnose leaf blight, anthracnose stalk rot, *Aspergillus* ear and kernel rot, and *Curvularia* leaf spot
Sorghum	Anthracnose, *Fusarium* head blight, root and stalk rot, grain storage mold, seedling blight and seed rot, and head smut
Oat	Anthracnose, *Fusarium* foot rot, head blight, root rot, seedling blight, covered smut, loose smut, and Victoria blight
Barley	Anthracnose, common root rot, crown rot and seedling blight, ergot, kernel blight, and net blotch

international spread (Agarwal and Sinclair 1996). The seed-borne diseases during germination and seedling mortality are main reason for poor crop that results in low production (Khalid et al. 2001); failure of plant development; crop yield and construction of plant toxin from contaminated seed (Table 12.1) (Williams and McDonald 1983; Kubiak and Korbas 1999; Dawson and Bateman 2001).

There are numerous responsible reasons for seed contamination, infection, and development of pathogenic fungus, like nonspecific agronomical practices, lack of seed storage amenities, and adverse climatic condition during crop production (Janardhana et al. 1999). Fungal pathogens are main responsible reason to eliminate the production of agricultural crops for human and animal feed. Nutritional quality and seed quantity or quality are reducing highly due to association of several fungal pathogens. Many fungal pathogens are associated with agricultural crops; among them nearly 300 fungal microflora are associated with an approximate of 25% of world crop production that produces harmful mycotoxins for human and animals. Several seed-borne fungal pathogens and their occurrence are important for predicting the infection range after harvest, colonization, and subsequent deterioration of the seeds. To solve the above problems of toxic seed-borne fungi, the main focus is to know the diversity of fungus, their occurrence, and their management study. This chapter provides particular information on diversity of fungal pathogen associated with seed.

The germination of seed and standing field crops is influenced by majority of fungal seed-borne pathogens. Due to the infection of fungal pathogen, seeds become dormant, or they do not germinate and transmit diseases from generation to generation by infected seed material (Islam and Borthakur 2012). Seed-borne fungal microflora may be present externally or internally and attached to seed as impurities (Singh and Mathur 2004). Some saprophytes and minor fungal pathogen are also responsible to reduced quantity or quality and market values of seed (Elias et al. 2004; Al-Askar et al. 2012).

The major seed-borne fungal genera of cereal crops including *Tilletia*, *Ustilago*, *Bipolaris*, *Fusarium*, *Alternaria*, *Drechslera*, *Stemphylium*, *Curvularia*, *Cladosporium*, *Rhizopus*, *Aspergillus*, and *Penicillium* were described throughout the world (Table 12.1) (Nirenberg et al. 1994; Glazek 1997; Fakhrunnisa et al. 2006; Rehman et al. 2011; Suproniene et al. 2011).

Low germination percent, discoloration and shriveling, development of plant diseases, distribution of pathogen to new areas, introduction of new strains or physiologic races of the pathogen along with new germplasm from other countries, toxin production in infected seed etc. occur due to infection of fungal pathogen in seed (Du et al. 2001; Rajput et al. 2005; Niaz and Dawar 2009). Different geographic areas or some environmental factors including relative humidity, temperature, rainfall, and moisture content are responsible for the degree of infection in seeds. It is necessary to identify the yield losses caused by seed-borne pathogens, for example, the loss reported in wheat ranges between 15 and 90% (Wiese 1987). For the cultivation and production of increased seed of different agricultural crops, it is necessary to identify the health and potential for high germination of seeds.

12.2 Important Seed-Borne Fungal Plant Pathogens

12.2.1 *Fusarium*

Fusarium belongs to family *Nectriaceae* and it is a large genus of filamentous fungus. It is a cosmopolitan soil fungus, and its species are responsible for seed-borne diseases in many crops. *Fusarium* species occur in many economically important plants like cereals, and ornamental plants and cause losses in crop yield (Appel and Gordon 1996).

12.2.1.1 Distribution

Fusarium has a worldwide distribution and includes vital range of economically important seed-borne fungal pathogens, viz., *Fusarium acuminatum*, *F. avenaceum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. nivale*, *F. poae*, *F. sambucinum*, *F. sporotrichioides*, *F. anthophilum*, *F. oxysporum*, *F. proliferatum*, *F. semitectum*, *F. solani*, and *F. verticillioides* (Nagaraja et al. 2016). Species of *Fusarium* are distributed in different geographic region of world but some species of *Fusarium* are found associated with specific plant species with specific geographic region or climatic zone of India and world.

The maximum distribution of *Fusarium* spp. was found in the vascular system. But any other growth stages of the host plants may also be infected by *Fusarium oxysporum*. Among them tomato, tobacco, legumes, cucurbits, sweet potatoes, and banana are highly susceptible to *Fusarium*, but it also infects some other agricultural crops. Vascular wilt is the most important symptoms of *Fusarium* spp. Wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off are some other core symptoms of *Fusarium oxysporum*, *Fusarium oxysporum* is split into divisions called formae speciales. There are over 100 formae speciales divisions with the different races.

12.2.1.2 Biology

Fusarium sp. comes under the *Sordariomycetes* class of the *Ascomycota* based on the structure of conidiogenous hyphae. Microconidia, macroconidia, and chlamydospores are asexual spores reported from *Fusarium* species (Nelson et al. 1983). Monophialides and sporodochia produce conidia which are loosely scattered on the mycelium (Griffin 1994). The microconidia of *Fusarium* are predominantly uninucleate and germinate poorly ranging from 1 to 20% (Ebbole and Sachs 1990). Comparative to microconidia, the macroconidia reproduce fungus efficiently because they are abundant, multinucleate, and germinate rapidly. The vegetative hyphae of *Fusarium* produce chlamydospores which are accessory spores, result of asexual reproduction (structural modification) and these remain viable in unfavorable conditions. The conidial cell which possesses a thick wall, mainly consisting of newly synthesized cell wall material, may result into chlamydospores due to structural modification (Schippers and van Eck 1981). It's function is primarily survival in seed/soil.

12.2.1.3 Symptoms

Fusarium is popularly known for causing *Fusarium* wilt in many agriculturally important crops. Among them *Fusarium* wilt of banana is caused by fungus known as *F. oxysporum* f. sp. *cubense* (*Foc*).

The starting or primary symptoms occur on leaves and seem like vein clearing of younger leaf, yellowing of the lower leaves, dropping down of lower leaves, stunting of plant, and finally death of the plant. The distinct symptoms can be seen on older plants between the blossoming and fruit maturation stages (Priest and Campbell 2003). Each forma specialis within the species is host-specific and produces different symptoms.

Sweet potato is affected by two *Fusarium* species; *F. oxysporum* f. sp. *batatas* is more common. Both species show symptoms on leaf like leaf chlorosis, leaf drop, and finally stunting of the plant growth. The disease caused by *F. oxysporum* f. sp. *batatas* in sweet potato transferred by soil and wounded material of plants. The *Fusarium* wilt disease of Canary Islands date palm and other propagated palms is caused by *Fusarium oxysporum* f. sp. *canariensis* and the spread of disease is found by contaminated seed, soil, and pruning tools. *F. oxysporum* f. sp. *cubense* causes Panama disease on banana. It is distributed in all over the world where bananas are grown such as Africa, Asia, and Central and South America. The Panama disease of banana is found in all age groups of plant and transferred mostly by soil. Wilting and yellowing of the leaves are the main symptoms of the disease (Katona and Kaminski 2002). *F. oxysporum* f. sp. *lycopersici* causes vascular wilt in tomato. The primary symptoms of the disease occur as yellowing of leaves and drooping on one side of the plant. It also causes leaf wilting, plant stunting, browning of the vascular system, and loss of fruit production. *F. oxysporum* f. sp. *melonis* attacks on muskmelon and cantaloupe. It causes damping-off in seedlings and causes chlorosis, stunting, and wilting in old plants. Necrotic streaks can appear on the stems. Apart from wilt disease, *Fusarium* spp. cause many important plant diseases, viz., bakanae disease of rice, head blight of wheat, Panama disease of banana, etc.

12.2.2 *Tilletia*

Karnal bunt and smut are popularly known diseases of wheat spread all over the world. Infected grain of wheat with Karnal bunt gives smell but infected grains are nontoxic for human beings and animals. Three diseases including common bunt caused by *Tilletia tritici* and *T. laevis*, loose smut caused by *Ustilago tritici*, and flag smut caused by *Urocystis tritici* occur in Australia and other wheat-growing regions of the world. Another two bunt diseases including Karnal bunt caused by *T. indica* and dwarf bunt caused by *T. controversa* are important diseases and restricted throughout the world and come under quarantine regulations.

12.2.2.1 Distribution

Karnal or partial Bunt disease of wheat was first reported from Karnal (India) in 1931 so it is called as Karnal bunt. The causal organism of Karnal bunt is *Tilletia indica* Mitra (syn. *Neovossia indica* (Mitra) Mundk.). It is distributed in Northwest India and nearby area of Pakistan and Afghanistan (Wiese 1987). The disease is reported not only from India but also from Iran, Iraq, Nepal (USDA Karnal Bunt Manual 2003), Mexico, the Southwestern United States, and South Africa (Fuentes-Davila 1996; Crous et al. 2001). Further, disease was detected in Texas and parts of California and also testified positive with Karnal bunt in shipments from Lebanon and Syria (USDA Karnal Bunt Manual 2003).

12.2.2.2 Biology

Contaminated seed and farm equipments are the main sources for disease distribution. The disease is also spread through wind to short distance area especially followed by burning of wheat straw by farmers. Halasz et al. in 2014 experimentally proved the dispersal of *Tilletia* by air. During the experiment, they observed that the highest concentration of teliospores was found above burning wheat field with contaminated wheat grains. Further, the above study concluded by many scientists that post-harvest disease development occurs due to teliospores distributed through air. The pathogen can remain in dormant condition in infected wheat grain for a long time and become active or germinate when favorable conditions arise (U.S. Department of Agriculture 2007). During the favorable condition, spores germinate and pathogens infect all wheat flowers and a mass of spores can be seen in whole kernels and embryo but usually the entire kernel is not affected.

Under favorable environmental conditions, teliospore comes in contact with suitable host and by germination produces promycelium. After complete development of promycelium, around 65–180 primary sporidia can be seen. The secondary sporidia develop from bud of primary sporidia and from mycelium of fungal threads. The secondary sporidia are the main infectants responsible for disease development in young plant at flowering stage by ovary wall. The entry of secondary sporidia in host plant is achieved by penetrating through germ tubes. After the penetration, the sporidia come in contact with kernels and replace healthy tissues of kernels with

vast number of teliospores. The teliospores remain as inactive spores in soil during harvesting practices and transfer from one field to another by wind and farming tools. These resting teliospores are responsible for further disease development in the next crop season.

12.2.2.3 Symptoms

Early stage of disease development bunts symptoms cannot be seen, but in severe conditions after disease development, it can be seen on crop, and conspicuous smell can be felt from infected wheat grains. The identification of bunt disease can be done on the bases of morphological study of teliospore and symptoms of infected seeds and by molecular techniques.

12.2.3 *Alternaria*

The *Alternaria* belongs to family *Pleosporaceae* and comes under the *Ascomycetes* group which has many saprophytic and parasitic fungal species. It is also well-known for its extremely destructive behavior for plant and human beings. *Alternaria* is frequently found in various economically important and cultivated families including Cucurbitaceae, Brassicaceae, and Solanaceae, but cruciferous crops like broccoli, cauliflower (*Brassica oleracea* L. var. *botrytis* L.), field mustard, turnip (*B. rapa* L. (synonym: *B. campestris* L.), Chinese mustard or leaf mustard (*B. juncea*), Chinese or celery cabbage (*B. pekinensis*), cabbage (*B. oleracea* var. *capitata*), rape (*B. campestris*), and radish (*Raphanus sativus*) are highly infected with *Alternaria* comparative to other crop.

12.2.3.1 Distribution

Species of *Alternaria* have cosmopolitan distribution especially tropical and subtropical region of the world. It has a wide range of disease distribution in various economically important and popular commercially cultivated crops including cereals, legumes, and oilseeds and a large number of post-harvest crops. Generally, early blight of crops is caused by *Alternaria* and its strains and it damages widespread tropical crops.

12.2.3.2 Biology

Alternaria produces asexual conidia/spores from conidiophores, size ranging from 160 to 200 μm long. The sporulations occur between the range of 8 and 24 °C under aseptic condition, and mature spores develop after 14–24 h. The ideal time and temperature for sporulation are 12 and 14 h and 16 and 24 °C, respectivily. Some environmental factors like moisture, rain, and humidity and time ranging from 9 to 18 h are important requirements for infection for majority of the species (Humpherson-Jones and Phelps 1989). Many workers experimentally proved that continuous moisture for 24 h or more than gives confirmation of infection (Rangel 1945;

Chupp and Sherf 1960). To produce a large number of mature spores, the required condition is 91.5% relative humidity with 20 °C or higher temperature in 24 h (Humpherson-Jones and Phelps 1989).

The seed coats infected with pathogen are primary source for disease or pathogen transport. Wind, water, agricultural tools, and animals are some factors responsible for spores' distribution. After harvesting, the pathogens remain in dormant condition on susceptible weed and other crops which are the major source of *Alternaria* species (Rangel 1945; Chupp and Sherf 1960; Maude and Humpherson-Jones 1980a, b). Earlier, it was studied that pathogen can be found viable on detached infected leaves of oilseed rape and cabbage. For oilseed rape, this was up to 8 weeks and for cabbage up to 12 weeks (Humpherson-Jones 1989). This type of spread is likely to occur in seedling beds as well, and seedlings from infected seed beds can carry the inoculum to the field (Rangel 1945).

12.2.3.3 Symptoms

Alternaria causes blight disease resulting in 32–57% average fruit yield loss on crops (Conn and Tewari 1990). Blight is the most common and dominant disease which shows irregular, often circular brown- to dark brown-colored spots on the leaves with concentric lines inside the spots. Generally, small circular spots join together and become large in size and lead to leaf blight. These small spots sometimes can be seen on pods and tender twinges (Valkonen and Koponen 1990).

12.2.4 *Curvularia*

12.2.4.1 Distribution

Curvularia is a facultative plant pathogen which belongs to family *Pleosporaceae* that comes under *Ascomycetes* group. It is a phytopathogenic saprophytic fungus which spreads throughout tropical and subtropical regions and mostly found in soil, air, organic matter, plants, and animals (Travis et al. 1991). It was studied that nearly 30 species of *Curvularia* are found in anamorphic states of the *Loculoascomycetes* genera *Cochliobolus* and *Pseudocochliobolus* (Rossman et al. 1987). The majority of diseases caused by *Curvularia* species have been reported from India and out of the country including the United States, Brazil, Japan, and Australia.

12.2.4.2 Biology

It is broadly spread all over the world but especially predominated in tropical and subtropical regions (da Cunha et al. 2013). The inoculum surviving in the soil is the primary reason for the infection that is caused by air-borne conidia and ascospores. The inoculum requires 24–30 °C temperature in aseptic condition for growth, but it will become dead on temperature exposure at 59 °C for 1 min or 55 °C for 5 min. The susceptible condition is required for successful infection when the host plant comes in contact with wet surface for 13 h.

12.2.4.3 Symptoms

Curvularia species cause discoloration of the aleurone and starch layer, and the hulls become brown (Tullis 1936; Umehara et al. 1979; Taketani and Yage 1982). A close association exists between discoloration of glumes, empty glumes, and kernels. When an empty glume dies, severe smudge of the kernels is observed (Taketani and Yage 1982). In favorable conditions, *Curvularia* causes discoloration on rice grains, and a few of them mold the grain (Groves and Skolko 1945; Bugnicourt 1950; Padwick 1950; Wei 1957). In severe infections, *Curvularia* spp. may cause seedling blight and weakening of the seedling (Ou 1985).

12.2.5 *Penicillium*

Johann Heinrich Friedrich Link (1809) first time reported about the genus *Penicillium* in his work *Observationes in ordines plantarum naturales*, whereas word *Penicillium* was derived from penicillatis referring to "pencil-like." It is also used as an antibiotic to remove some bacteria.

12.2.5.1 Distribution

Penicillium species are globally distributed in soil and infected seeds; it requires cool and moderate climate for infection. The pathogen is commonly found wherever decayed organic material is available. *Eurotiales* are the most known mold or sac fungi which are represented by saprophytic fungus *Penicillium*. In America, it is popularly known as mold fungus and responsible for food spoilage, especially species of subgenus *Penicillium*. Many species of *Penicillium* produce extremely toxic compounds which restrict the growth of bacteria. The spores of *Penicillium* species are available in open air and dust such as homes, offices, and public buildings. From the open environment, the spores are easily distributed from one place to another place and grow simply in indoors.

12.2.5.2 Biology

Penicillium have branched mycelium with multinucleate, septate, usually colorless hyphae. The branched mycelium produces conidiophore which independently yields conidiospores. These conidiospores are primary source of fungal spread or distribution.

They multiply by sexual reproduction in which two nuclei of ascogonium and antheridium fuse together and produce ascospores. The asci which are distributed irregularly, contains generally eight unicellular ascospores.

12.2.5.3 Symptoms

The *Penicillium* spores are dispersed through air and grow on living carbonic matter and stored seeds which have low humidity, and sufficient moist condition, favorable for spore development. Generally, the spores have green color, but sometimes, they have blue color which grow on old bread and create blue fuzzy texture. Some

species attack on temperate and tropical fruits and bulbus like *P. expansum* infects apples and pears fruit; *P. digitatum* infects citrus fruits; and *P. allii* is parasitic on garlic.

12.2.6 *Aspergillus*

Aspergillus is a common fungus of mold group which belongs to family *Trichocomaceae*. Pier Antonio Micheli first time studied and cataloged this fungus in 1729. *Aspergillus* is an asexual spore-bearing fungus which contains more than 200 species and commonly found in seed coat of infected seeds. Prakash and Jha (2014) studied about the reproductive structure of *Aspergillus* in which conidiophore bears round cluster of conidial chains at the end.

12.2.6.1 Distribution

Species of *Aspergillus* are available generally in soils as saprophytes and widely distributed in subtropical regions at 25–35 degrees latitude (Robertson et al. 2013). Particularly *Aspergillus niger* can tolerate temperature nearly about 42 °C, but 30–37 °C is the optimum temperature for maximum growth. Below 15 °C the fungal growth becomes slow or stops (Zhang and Yang 2000). *Aspergillus niger* requires 8 pH for maximum growth, but in low pH culture medium, it produces several kinds of acids (Desjardins et al. 2002).

12.2.6.2 Biology

Aspergillus is a heterothallic fungus which produces it's spores by two different methods. One is sexual method in which two different nuclei fuse together and produce a new spore body, while another is self-fertilization (in homothallic species), in which both nuclei come from same fruiting body and form a new one. *Aspergillus* is defined as a group of conidial fungi but belongs to *Ascomycota* with DNA evidence.

12.2.6.3 Symptoms

Aspergillus species grow rapidly on seeds and other stored foods in the presence of high moisture, and it spreads by aerial dispersion. *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Aspergillus niger* are some common seed-borne *Aspergillus* species.

12.3 Role of Indian Type Culture Collection (ITCC) in Seed Pathology

Fungal plant pathogens are potential biotic threats to crop production system. The reliable identification and documentation of fungal pathogens require type strains of the particular causal species or pathovars and subspecies. Generally, researchers working on fungal pathogens procure the reference strains from abroad culture

collection centers in order to do confirmation of their strains, which is time-consuming as well as cost-incurring. To address this issue, a repository of major microbial plant pathogens along with their identification and supply services has been established as Indian Type Culture Collection (ITCC) in the Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, in 1936. At present 4000 fungal cultures are available with ITCC. ITCC is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM, registration number 430). Central Insecticide Board, Directorate of Plant Protection, Quarantine & Storage, Ministry of Agriculture and Farmers' Welfare, Government of India, had made it mandatory to deposit the bio-agents at ITCC before they are released. Also, the accession number of ITCC is a prerequisite for publication.

Culture collection centers are worldwide encouraged to create novel and better techniques for bio-prospecting of novel microorganisms, carry out molecular sequence analysis, define phylogenetic relationships, execute taxon-based research on population structure of natural consortia and pools of microorganisms, and monitor and track genetically engineered microorganisms that have been released for use in the environment and industry, and many identified species/strains of microbes related to seed-borne plant pathogens available with ITCC need to be explored for disease management research (Table 12.2).

Table 12.2 Cultures available in ITCC which are isolated from seeds

S. no.	Name of seed-borne plant pathogens	ITCC no.
1.	***Absidia corymbifera***	3480
2.	***Actinomucor elegans***	4252
3.	***Alternaria alternata***	3467, 7172
4.	***Aspergillus amstelodami***	6732, 3166
5.	***Aspergillus clavatus***	2397, 3473, 6347, 6352, 6353, 6363, 6972
6.	***Aspergillus fumigatus***	3474, 7263
7.	***Aspergillus japonicus***	2396
8.	***Aspergillus niger***	6348, 6349, 6738
9.	***Aspergillus ochraceus***	1740, 2391, 6350, 6358, 6361, 6743
10.	***Aspergillus quercinus***	2929
11.	***Aspergillus sydowii***	2631
12.	***Aspergillus ustus***	2912
13.	***Aspergillus versicolor***	1861
14.	***Bipolaris hawaiiensis***	6710
15.	***Bipolaris spicifera***	3250
16.	***Chaetomium brasiliense***	7379
17.	***Chaetomium funicola***	2936, 5137
18.	***Chaetomium globosum***	1813, 2936, 2937, 2938, 3477, 7370
19.	***Chaetomium megalocarpum***	2934, 2935, 7373, 7380, 7384
20.	***Cladosporium cladosporioides***	6579, 7073

(continued)

Table 12.2 (continued)

S. no.	Name of seed-borne plant pathogens	ITCC no.
21.	***Cladosporium oxysporum***	2795
22.	***Cladosporium sphaerospermum***	6934
23.	***Corticium rolfsii***	6866
24.	***Cunninghamella echinulata***	6733
25.	***Colletotrichum sublineolum***	6750
26.	***Curvularia geniculata***	3171
27.	***Curvularia lunata***	4613, 6081
28.	***Curvularia lunata* var. *aeria***	2979, 6935
29.	***Curvularia senegalensis***	4740
30.	***Exserohilum rostratum***	2048
31.	***Fusarium acuminatum***	6867
32.	***Fusarium chlamydosporum***	3451, 6936
33.	***Fusarium compactum***	4457
34.	***Fusarium oxysporum***	2389, 2969, 6745, 7333, 7367
35.	***Fusarium pallidoroseum***	6746
36.	***Mucor hiemalis***	2406
37.	***Penicillium brevicompactum***	4328
38.	***Penicillium chrysogenum***	2844, 2981, 4329
39.	***Penicillium citrinum***	2409, 6588, 6589
40.	***Penicillium implicatum***	3984
41.	***Penicillium janthinellum***	2408
42.	***Penicillium puberulum***	3643
43.	***Phoma betae***	2238
44.	***Rhizopus stolonifer***	2412
45.	***Scopulariopsis brevicaulis***	4523
46.	***Stachybotrys atra***	4736, 6207
47.	***Thanatephorus cucumeris***	5592, 6180
48.	***Trichoderma longibrachiatum***	6999
49.	***Trichothecium roseum***	4524, 4611
50.	***Tritirachium oryzae***	4741
51.	***Ulocladium botrytis***	2677
52.	***Xylochia oryzae***	6120

http://www.iari.res.in/files/Divisions/PPathology/ITCC_catalogue_1936-2016-16092016.pdf

12.4 Conclusion

The inner and outer parts of seeds represent an ecological niche where numerous species of fungal pathogens live. These ubiquitous fungi have an effect in the way plants interact with their environment. It appears that most seed-borne fungi are pathogenic but some also help plants to cope with biotic and abiotic stress situations. Therefore, seed-borne fungi may have an important role in the adaptation of plants to some particular environments. In addition, they represent a group of many

economically important phytopathogens which cause huge economic loss. In order to avoid losses due to seed-borne diseases, the approaches suggested are (1) seed certifications schemes, (2) establishment of an effective quarantine control system, (3) establishment of seed health testing, and (4) research units and training centers. All imported seed consignments should be tested in the laboratory, and the growing-on tests in equipped greenhouses are necessary.

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Diversity of Seed-Borne Bacterial Phytopathogens

13

S. Umesha

Abstract

Globalization of world agriculture increases easy movement and genetic diversity of phytopathogens in seeds across the countries through long distance of exchange. Early detection, diversity, and management of seed-borne bacteria are predominantly essential, as often infected seeds appear symptomless making pathogen's detection and study of their diversity difficult. To address these issues in bacterial seed-borne pathogens such as *Acidovorax avenae*, *Clavibacter michiganensis*, *Curtobacterium flaccumfaciens*, *Pseudomonas syringae*, *Xanthomonas campestris*, and *X. oryzae* pv. *oryzae*, several assays and techniques have been developed. In the last decades, seed-borne phytopathogens have been differentiated based upon restriction fragment length polymorphism (RFLP) analyses, a random amplified polymorphic DNA fragment (RAPD), DNA-DNA hybridization, and pulsed-field gel electrophoresis. Genetic diversity based on the small ribosomal subunit sequence (16S rDNA gene sequence) has been one of the most common methods for phylogenetic analyses of seed-borne pathogens. However, these methods are problematic because of their lack of resolution when comparing closely related organisms as well as recombination and lateral gene transfer events. Multilocus sequence analysis/typing (MLSA/T) and repetitive element polymerase chain reaction (rep-PCR) often overcome the problems of the 16S rDNA-inferred phylogenies and have been proven to be more reliable than the 16S rDNA in the genera. At present the best way of genetic analysis concentrated on sequencing specific genes, comparative whole genome sequencing, and next-generation sequence analysis (NGS) of closely related isolates. Sequencing of

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phytopathogenic bacteria represents a powerful tool for rapid identification and to study the genetic diversity of genes involved in host specificity and virulence of seed-borne pathogens.

13.1 Introduction

A seed is the basic unit in crop production technology. Seeds are recognized containers of embryos of a new generation, carrier for spread and regeneration of plant life, as well as the significant contributor for substantial agriculture. Consequently, pathogen-free healthy seeds play a vital role for desired plant population and serve as the backbone for good economic harvest. The seeds carry a heavy load of microorganisms which are capable of causing severe diseases and thus a considerable loss of yield. Even though seed production has been motivated to semiarid regions to escape seed-borne pathogens, seed-borne bacterial diseases continue to be problematic and cause significant economic losses worldwide (Gitaitis and Walcott 2007). The bacterial phytopathogens enter into the seed coat, cotyledons, and embryonic parts at storage condition. Bacterial seed-borne pathogens with the seed cause several types of abnormalities like seed damage, reduction in germination, seed discoloration, seed biodeterioration, seed poisoning, etc., and such infected seeds are poor in quality for consumption (Binyam 2015). Infested seeds are responsible for the re-emergence of diseases of the past, the movement of pathogens across international borders, or the introduction of diseases into new areas. Considerable attention has been paid to improve the sensitivity and selectivity of seed health detection and diversity. In today's global economy, seed accounts more than ever for the movement of plant pathogens across vast distances, natural barriers, and political borders. Seed-borne bacterial pathogens are of particular concern because, unlike seed-borne fungi, strategies for the management of bacterial diseases are inadequate, especially under limited and antiquated chemical options available (Gitaitis and Walcott 2007).

Seeds growing in agricultural field under natural circumstances are exposed to numerous dissimilar species of pathogen. Every species exists as a population of several individuals between which there are almost invariably a number of genetic variations. The truth that crops are exposed to pathogen populations which are genetically diverse is an important contemplation in the management of several seed-borne bacterial diseases. Each bacterial phytopathogen varies in its prospective range of host species, capacity to infect different genotypes of a crop, and adaptation to non-biological factors such as temperature, abiotic stress, and resistance toward chemicals/antibiotics which have diversity in agricultural fields. Each one of these factors might affect the course of a disease, whether as an infection of a single plant or as an epidemic in a crop or in a natural population of plants (Brown 1996). In the last few decades, molecular genetic diversity study has had a major impact on seed-borne bacteria.

13.2 Seed-Borne Pathogens

Almost 90% of the crops cultivated are propagated by seeds (Neergaard 1977). Seeds form the major input in crop strategy, and the quality of the seeds plays a vital role in determining the yield and losses of sustainable crop production. Seed-borne diseases caused by bacteria, fungi, viruses, nematodes, and phytoplasma have gained economical significance accounting for major crop losses worldwide every year. The major concern of seed-borne diseases is the dissemination of pathogens from generation to generations which makes it problematic to completely eradicate the pathogens. Healthy seeds are the basic requirement for producing good food needed for human survival and maintenance. Dispersal of plant pathogens via seeds has assumed more importance than other modes of dispersal, as infected seeds have the potential to become the primary source of carrying pathogen inoculum for subsequent crops. Several diseases transmitted through seeds damage the economies, as a result of huge losses in numerous crops. Hence, foremost importance is being given for the detection, identification, and differentiation of the pathogens present in seeds using sensitive techniques.

Seed occupies only a small niche in the overall agricultural economy, and the losses presented to the agricultural economy by seed-borne diseases are presented in many forms. The seedlings from infected seeds usually are abnormal seedlings, poor seedling vigor, reduced germination and very important that seed-borne pathogens may play an important role in the economy of the agriculture and food security to the world (Perelló et al. 2013). Absence of suitable control methods helps in the carrying of pathogens by seed to the uninfected areas and causes economic losses. Apart from seed, secondary inoculums are also carried through wind, irrigation water, rain, insects, animals, and even humans spread to long distance from the original infection source. The disease depends on the ability of pathogen to survive outside prior to infection, multiply in the host, disperse, and transmit to new ecological niche (Darrasse et al. 2010).

Seed-borne pathogens are microorganisms that negatively affect plant survival, growth, and/or reproduction. Such microbes have great taxonomic breadth, including obligatory pathogenic fungi, bacteria, viruses, and water molds (*Oomycetes*) (Alberts et al. 2002). Disease development and dissemination of pathogens from contaminated seed lots can be predicted using formula that take into account inoculum density and environmental pressures. In general, seeds infested with bacterial pathogens are distributed within a Poisson distribution (Gitaitis and Walcott 2007). Bacteria pathogenic to plants are responsible for devastating losses in agriculture. The use of antibiotics to control such infections is restricted in many countries due to worries over the evolution and transmission of antibiotic resistance. The advent of genome sequencing has enabled a better understanding, at the molecular level, of the strategies and mechanisms of pathogenesis, the evolution of resistance to plant defense mechanisms, and the conversion of non-pathogenic into pathogenic bacteria (Jackson 2009).

13.3 Common Seed-Borne Bacterial Pathogens

Seed-borne pathogens are a continuing problem and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas. The most important bean bacterial pathogens transmitted by seeds, *Pseudomonas savastanoi* pv. *phaseolicola*, *Pseudomonas syringae* pv. *syringae*, *Xanthomonas axonopodis* pv. *phaseoli*, and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, are widespread and destructive diseases in bean-growing regions (Coyne and Schuster 1974; Yoshii et al. 1978; Schwartz and Galvez 1981). For the bacterial brown spot caused by *P. savastanoi* pv. *syringae*, symptoms are similar to young halo blight lesions after initial infection. The symptoms appear as small water-soaked spots most visible on the underside of young foliage. When the lesion matures, it typically develops a "brown spot" appearance, and dead tissue in the center may fall out, producing a shot-hole appearance (Saettler 1991). Common bacterial blight, caused by *X. axonopodis* pv. *phaseoli* and the brown-pigmented variant *X. axonopodis* pv. *phaseoli* var. *fuscans* (Schaad 1982), is a major worldwide seed-borne disease of bean, causing yield reductions from 10 to 40% in susceptible cultivars leading to economic losses of thousands of millions of US dollars (Wallen and Jackson 1975; Weller and Saettler 1980).

Following is the list of bacterial pathogens borne in true seeds in important crops:

1. Wheat: *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris* pv. *translucens.*
2. Maize: *Pantoea stewartii* subsp. *stewartii*, *Clavibacter michiganensis* subsp. *nebraskensis.*
3. Rice: *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, *Acidovorax oryzae.*
4. Bean: *P. syringae* pv. *phaseolicola*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Xanthomonas campestris* pv. *phaseoli*, and *X. fuscans* var. *fuscans.*
5. Soybean: *P. syringae* pv. *glycinea.*
6. Chickpea: *Rhodococcus fascians.*
7. Cereals and grasses: *Rathayibacter* sp.
8. Alfalfa: *C. michiganensis* subsp. *insidiosus.*

Examples of some important seed-borne bacterial diseases are bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* in rice, basal glume blotch caused by *P. syringae* pv. *syringae* in wheat, Goss's bacterial wilt and blight caused by *Clavibacter michiganensis* subsp. *nebraskensis* in maize, bacterial blight caused by *P. syringae* pv. *glycinea* in soybean, and black rot caused by the bacterium *X. campestris* in crucifers and carrot.

13.3.1 *Xanthomonas oryzae* pv. *oryzae*

Xanthomonas oryzae causes bacterial blight disease which is considered as the most devastating disease of rice worldwide (Zhang and Wang 2013). *X. oryzae* pv. *oryzae*

is a gram-negative bacterium which is non-spore-forming and rod-shape in structure. The colonies are smooth, circular, convex, and light yellow, and it produces water-soluble yellow pigment. *X. oryzae* pv. *oryzae* survives primarily in rice stubble and also survives for short periods on infected seed and in soil (Mew 1992).

X. oryzae pv. *oryzae* infects the rice at the leaf tip hydathodes and leaf margin. It multiplies in intercellular spaces of the underlying epithelial cells. After entry, it spreads into the plant through the xylem or through wounds or openings caused by emerging roots at the base of the leaf sheath. Within the xylem, *X. oryzae* pv. *oryzae* most probably interacts with the xylem parenchyma cells, and then it moves vertically through the leaf via the primary veins and may also progress laterally through commissural veins. Within a few days, bacterial cells expel the EPS (extracellular polysaccharide) to fill the xylem vessels and ooze out from hydathodes, forming beads or strands of exudates on the leaf surface. This is a characteristic sign of the *X. oryzae* pv. *oryzae* disease incitant and a source of secondary inoculum (Nino-Liu et al. 2006). As the bacterium develops in the plant, it can also spread into the mesophyll. Bacterial leaf blight is favored by warm temperatures (25–30 °C, 77–86 °F), high humidity, and deep water. The disease is more prevalent in wetland areas where these conditions often occur.

The primary symptoms caused by *X. oryzae* pv. *oryzae* are leaf blight and wilt. Leaf blight is the most common symptom, which generally occurs at the maximum tillering stage. It begins as water-soaked stripes on the leaf blades. The stripes increase in length and width, become yellow and then white, and may combine to cover the entire leaf blade. Drops of ooze may be observed on young lesions. Bacteria may also "ooze" out of water pores on hydathodes with bacterial blight. Older infected leaves may appear grayish due to the growth of saprophytic fungi. Small, circular lesions with water-soaked margins may also form on the glumes. These plants produce fewer grains with poor quality. The wilt syndrome is also known as 'kresek phase' and is the most destructive manifestation of the disease which occurs from the seedling to the early tillering stage. The leaves of wilted plants roll up, are grayish green in color, and later turn yellow to straw color. Plants that do survive are stunted and yellow in color, and the entire plant generally dies.

The bacterial blight disease is controlled by spraying streptomycin and copper oxychloride solution which completely inhibits the growth of bacterium (Tagami and Mizukami 1962). Seed disinfection with streptomycin mixture was found to be effective which eradicates the pathogen in seeds (Srivastava 1972). Bleaching powder containing 30% chlorine (2 Kg/ha) also reduced the bacterial blight lesion in rice (Chand et al. 1979). Biological control is also carried out by using formulations Talc-A5 (*Bacillus firmus* E65, *Pseudomonas aeruginosa* C32b) which is found to be effective against bacterial leaf blight (Yadi et al. 2013).

Xanthomonas oryzae pv. *oryzae* strain (XOO) was the first *Xanthomonas* genome to be sequenced from a species that infects exclusively monocotyledonous plants, in this case rice. This genome was found to be similar to the two previously sequenced *Xanthomonas* genomes (*Xanthomonas axonopodis* pv. *citri* strain 306 [XAC] and *Xanthomonas campestris* pv. *campestris* strain ATCC [XCC-ATCC]; in terms of gene content (more than 80% of XOO's genes have orthologs in the other two

species)), but whole-chromosome alignments showed substantial rearrangements compared to both XAC and XCC-ATCC. The fact that XOO contains more than twice the number of transposable elements than either XAC or XCC-ATCC (which already contain quite a few of these) suggests that these elements are pervasive in the *Xanthomonas* genus and that they play a major role in the rearrangements (da Silva et al. 2002). XOO was found to have 245 unique genes with respect to XAC and XCC-ATCC; among these were found a restriction-modification gene, a TonB-dependent siderophore receptor, genes for toxin production (e.g., *mlrB* and *rtx*), and a type III secretion system effectors (Setubal et al. 2005).

13.3.2 *Pseudomonas syringae* pv. *syringae*

The *P. syringae* pv. *syringae* and other species of *Pseudomonas* affect wheat and other crops resulting in major reduction in the grain yield. As a species, *P. syringae* causes diseases in a wide range of economically important plant species. *P. syringae* pv. *syringae* exhibits host specificity and infects only a limited number of plant species or even a few cultivars of a single plant species. About 50 pathovars are described for *P. syringae*, and 9 different species were identified based on DNA homology. The wheat disease "basal glume blotch" caused by *Pseudomonas* strains is classified as *P. syringae* pv. *atrofaciens* involved in leaf blight, and it is grouped under *P. syringae* pv. *syringae.* In other countries like New Zealand, Italy, the USA, Australia, Canada, South Africa, Argentina, and Pakistan, the occurrence of *P. syringae* pv. *atrofaciens* and/or pv. *syringae* has been reported only once (Valencia-Botín and Cisneros-López 2012; Xin and He 2013).

The disease caused by *P. syringae* pv. *syringae* in wheat is called dieback or leaf blight disease. On expanding leaves, it induces water-soaked spots which become necrotic turning tan-white from gray-green. *P. syringae* pv. *syringae* tends to be sporadic and its geographical distribution is limited. The *P. syringae* subsp. *syringae* is considered unique among *P. syringae* pathovars for its ability to cause disease in at least 180 plant species from several unrelated genera (Little et al. 1998; Valencia-Botín and Cisneros-López 2012).

Under favorable environmental conditions such as high humidity, heavy rainfall, and moderate temperature, *P. syringae* can multiply very aggressively in a susceptible host plant. During pathogenesis stage, host cells die and infected tissues lead to extensive necrosis. This pathogenesis mode is distinct from that of strictly other biotrophic pathogens, which obtain nutrients from living host cells without causing necrosis, and from that of strictly necrotrophic pathogens, which kill host cells during early stages of infection as the main strategy of obtaining nutrients (Xin and He 2013).

13.3.3 *Acidovorax avenae* subsp. *avenae*

Acidovorax avenae subsp. *avenae*, formerly *Pseudomonas avenae*, can cause diseases in many plants of economic importance, including rice, corn, oats, sugarcane,

millet, and foxtail. In rice, this pathogen can cause bacterial brown stripe and has been reported in many countries in Asia, Africa, America, and Europe. The symptoms start as brown stripes on the bottom of stem 5 days after emergence and frequently extend into the sheaths, spreading along the leaf midrib and throughout the seedling at the one-leaf stage. As *A. avenae* subsp. *avenae* is a widely distributed seed-borne pathogen of rice, the rice seeds contaminated with this pathogen are important sources of the primary inoculum and a means of dissemination of the pathogen to new areas (Walcott et al. 2003).

DNA fingerprinting by pulsed-field gel electrophoresis and repetitive extragenic palindromic (REP)-polymerase chain reaction (PCR) confirmed two distinct groups among *Acidovorax avenae* subsp. *citrulli* strains collected from a range of cucurbitaceous hosts in the USA, China, Taiwan, Thailand, Canada, Australia, Brazil, and Israel. The draft genome sequence of *Acidovorax avenae* subsp. *avenae* RS-1 comprises 5,522,282 bases, representing approximately more than 99.9% of the estimated genome of RS-1. The genome of this strain has a high G + C content (68.7%). A total of 5043 coding sequences (CDSs) were predicted. The chromosome has 3 rRNA operons and 43 tRNAs predicted by RNAmmer and tRNA scan. Furthermore, 90.4% of the open reading frames (ORFs) have orthologs in the reference strain *A. avenae* subsp. *avenae* ATCC 19860 (BLASTP value <1e-5), but 301 ORFs were not found in the released genomes of members of the genus *Acidovorax*. Interestingly, many of these ORFs are clustered together. The result suggested that these regions may be genomic islands in *A. avenae* subsp. *avenae* RS-1 (Xie et al. 2011).

13.3.4 *Clavibacter michiganensis* subsp. *michiganensis*

Clavibacter michiganensis subsp. *michiganensis*, causal agent of tomato bacterial canker, is a seed-borne pathogen and is considered one of the most destructive bacterial diseases of this crop (Figs. 13.1 and 13.2). Its movement over long distances is facilitated by traded seeds, which explains its distribution throughout all the tomato-growing regions of the world, but its spread differs widely among countries. However, it can also survive in plant debris and on volunteer plants or alternative hosts that can act as local sources of inoculum (de León et al. 2011). In general, this disease shows a typical start/stop pattern, with devastating outbreaks that cause major economic losses but appear unpredictably in time (Strider 1969). Unfortunately, resistant or highly tolerant cultivars are still not commercially available, and assays focusing on the chemical control of *C. michiganensis* subsp. *michiganensis* are scarce (Milijašević- Marčić et al. 2012).

The nucleotide sequence of the genome of *Clavibacter michiganensis* subsp. *michiganensis* was determined. The chromosome is circular, consists of 3.298 Mb, and has a high G+C content (72.6%). Annotation revealed 3080 putative protein-encoding sequences; only 26 pseudogenes were detected. Two *rrn* operons, 45 tRNAs, and 3 small stable RNA genes were found. The two circular plasmids, pCM1 (27.4 kbp) and pCM2 (70.0 kbp), which carry pathogenicity genes and thus are essential for virulence, have lower G+C contents (66.5 and 67.6%, respectively).

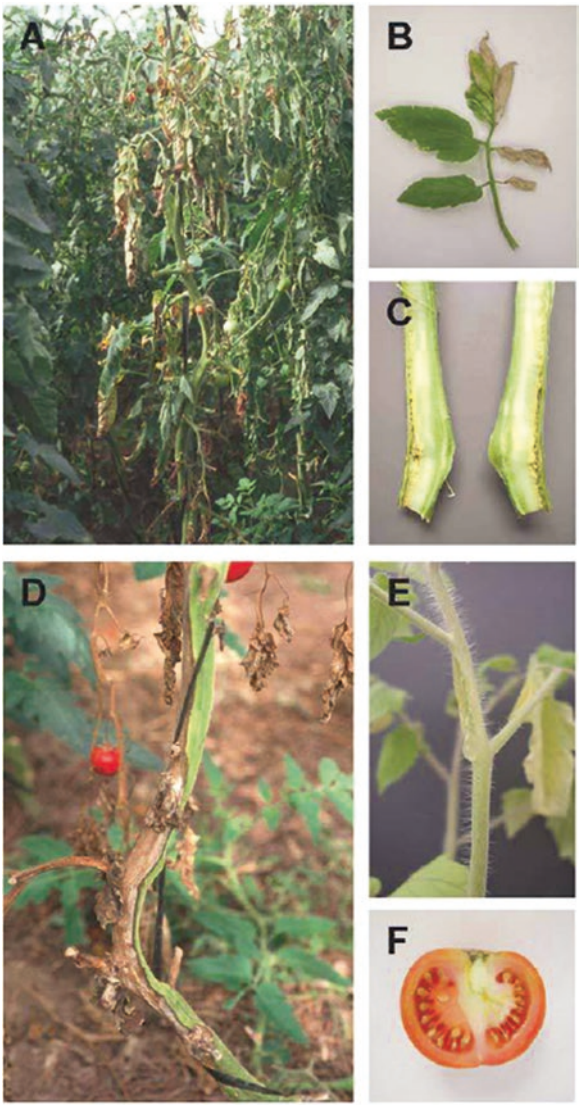

Fig. 13.1 Systemic infection on tomato plants by *Clavibacter michiganensis* subsp. *michiganensis*. (**a**) generalized wilting, (**b**) unilateral leaflet wilt and necrosis, (**c**) part of the vascular system invaded by the pathogen causing yellow-brown discoloration, (**d**) cankers on stems in later stages of disease development, (**e**) droplets of bacterial ooze observed when the stem splits open at the beginning of canker formation, and (**f**) pathogen reaching the fruit and infecting the seeds through the vascular tissues. Adapted from de Leon et al. (2011)

In contrast to the genome of the closely related organism *Clavibacter michiganensis* subsp. *sepedonicus*, the genome of *C. michiganensis* subsp. *michiganensis* lacks complete insertion elements and transposons. The 129-kb *chp/tomA* region with a low G+C content near the chromosomal origin of replication was shown to be necessary for pathogenicity. This region contains numerous genes encoding proteins involved in uptake and metabolism of sugars and several serine proteases. There is evidence that single genes located in this region, especially genes encoding serine

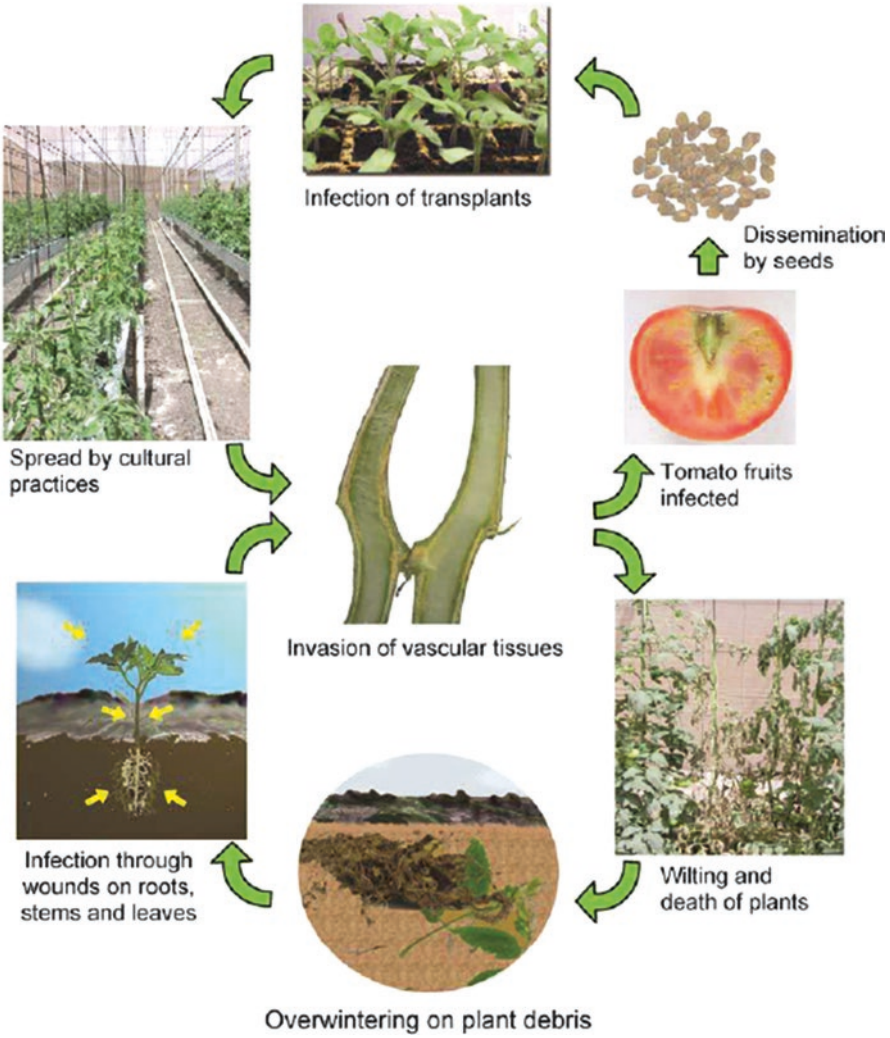

Fig. 13.2 Disease cycle of *Clavibacter michiganensis* subsp. *michiganensis*. Adapted from Eichenlaub et al. (2007)

proteases, are required for efficient colonization of the host (Gartemann et al. 2008). Although *C. michiganensis* subsp. *michiganensis* grows mainly in the xylem of tomato plants, no evidence for pronounced genome reduction was found. *C. michiganensis* subsp. *michiganensis* seems to have as many transporters and regulators as typical soil-inhabiting bacteria. However, the apparent lack of a sulfate reduction pathway, which makes *C. michiganensis* subsp. *michiganensis* dependent on reduced sulfur compounds for growth, is probably the reason for the poor survival of *C. michiganensis* subsp. *michiganensis* in soil (Gartemann et al. 2008).

13.3.5 *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

Curtobacterium flaccumfaciens pv. *flaccumfaciens* is a gram-positive bacterium and has re-emerged as an incitant of bacterial wilt in common (dry, edible) beans in western Nebraska, eastern Colorado, and southeastern Wyoming (Fig. 13.3). *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* is diverse phenotypically and genotypically and is represented by several different pathogen color variants (Agarkova et al. 2012). The population structure of different strains, including some isolated from alternate hosts, was determined with three molecular typing techniques, viz., amplified fragment length polymorphism (AFLP), repetitive extragenic palindromic polymerase chain reaction (rep-PCR), and pulsed-field gel electrophoresis (PFGE). All three typing techniques showed a great degree of population heterogeneity, but they were not congruent in cluster analysis of the *C. flaccumfaciens* pv. *flaccumfaciens* populations. Cluster analysis of a composite data set (AFLP, PFGE, and rep-PCR) using averages from all experiments yielded two distinct groups. Strains producing purple extracellular pigment were assigned to both clusters. Thus, *C. flaccumfaciens* pv. *flaccumfaciens* is diverse phenotypically and genotypically (Agarkova et al. 2012).

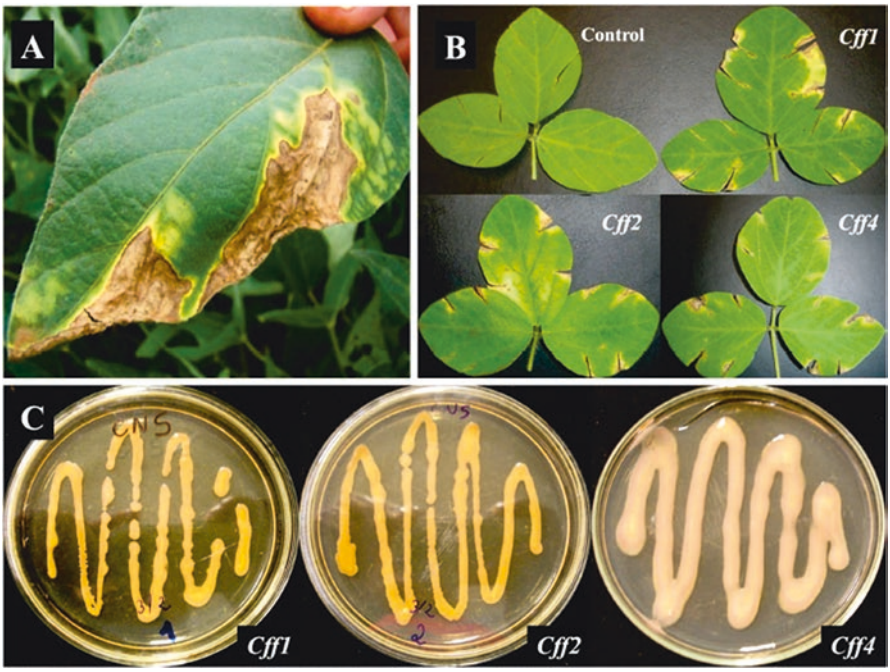

Fig. 13.3 (**a**) Systemic infection on plants by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. (**b**) Symptoms of scissor after pathogen inoculation. (**c**) Morphological colonies of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. Adapted from Soares et al. (2013)

13.3.6 *Pseudomonas syringae* pv. *tomato*

Pseudomonas is a bacterial genus that contains a wide range of animal and plant pathogens. *Pseudomonas syringae* pv. *tomato* was the first *Pseudomonas* phytopathogen to be sequenced (Nowell et al. 2016). A major result of this project was the identification of several clusters of genes that encode type III secretion system effector proteins (31 proteins confirmed and 19 predicted effectors). Most of these proteins are essential for pathogenicity. Orthologous genes are present in *Xanthomonas* and *Ralstonia* species, but many of them have not yet been confirmed to be expressed and secreted. Subsequent work on *P. syringae* strains has increased the list of predicted effector-coding genes to more than 200 (http://pseudomonas-syringae.org). A detailed analysis of additional genes in *Pseudomonas syringae* pv. *tomato* that are related to pathogenicity was done. A total of 298 genes were discovered. Of these, 96 are unique to *Pseudomonas syringae* pv. *tomato* with respect to *Pseudomonas aeruginosa* and *Pseudomonas putida*; thus, they are presumed to play a role specific to plant-*Pseudomonas syringae* pv. *tomato* interactions.

13.3.7 *Xanthomonas axonopodis* pv. *phaseoli*

X. axonopodis pv. *phaseoli* produces similar symptoms on leaves, pods, stems, and seeds. Small water-soaked spots are the first symptoms observed on leaves and appear within 4–10 days of infection. These spots enlarge and the center turns necrotic and brown. Areas around the lesion may become flaccid (Goodwin and Sopher 1994). The lesion is surrounded by a narrow band of bright yellow tissue. However, yellowed tissue is occasionally absent. Plant debris is probably more important as a source of inoculum in warmer climates where several susceptible crops can be planted in 1 year (Saettler 1989). However, *X. axonopodis* pv. *phaseoli* populations will drop sharply in plant debris as other microorganisms colonize and break down the material. If infected bean debris is tilled into the soil, it will greatly increase the rate of decay of the tissue and enhance the decline in *X. axonopodis* pv. *phaseoli* populations, since it appears to be unable to survive for long in soil as a free bacterium.

X. axonopodis pv. *phaseoli* can spread by planting infected seed. Plants grown from infected seed usually show lesions on the cotyledon or primary leaves. Bacteria in lesions, infected plant debris, and epiphytic populations can spread by splashing or wind-blown rain. Overhead irrigation can be very effective in the secondary spread of the bacteria. Surface irrigation water may also spread the bacteria. Infection occurs through natural openings and wounds. Severe epidemics can occur following storms with wind-blown rain, which can force the bacteria through openings, such as stomata, into the intercellular spaces. Wounds due to hail or insect feeding can create favorable sites for infection. Once inside the plant, *X. axonopodis* pv. *phaseoli* multiplies rapidly in the intercellular spaces, and it can take as little as 10–14 days from initial infection until secondary spread occurs (Saettler 1991).

Seed infection by *X. axonopodis* pv. *phaseoli* can be external or internal. Both hot water and dry heat have been successful in treating bean seeds for *X. axonopodis* pv. *phaseoli*. This involves incubating for either 20 min in 52 °C water or 23–32 h in 60 °C dry air at 45–55% RH. The latter treatment does not appear to affect seed viability. Treatment with an antibiotic such as streptomycin may be used to control external contamination with *X. axonopodis* pv. *phaseoli*, and streptomycin in polyethylene glycol may reduce, but not eliminate, internal populations of *X. axonopodis* pv. *phaseoli* (He 2010).

13.3.8 *Xanthomonas campestris* pv. *campestris*

This is a good example of how the study of closely related genomes can yield important insights (Bogdanove et al. 2011). *Xanthomonas campestris* pv. *campestris* 8004 and *Xanthomonas campestris* pv. *campestris* are two strains of the same species, but their pathogenicity properties in at least two hosts are remarkably different. As expected, their genome sequences are very similar. As in the case of *Xanthomonas oryzae* pv. *oryzae*, several chromosomal rearrangements were observed, with respect to both XAC and *Xanthomonas campestris* pv. *campestris*-ATCC. In fact, surprisingly, XAC and *Xanthomonas campestris* pv. *campestris*-ATCC have fewer rearrangements between them than *Xanthomonas campestris* pv. *campestris*-ATCC has with respect to *Xanthomonas campestris* pv. *campestris*-8004. Qian et al. (2005) identified 108 and 62 putative genes specific to *Xanthomonas campestris* pv. *campestris*-8004 and *Xanthomonas campestris* pv. *campestris*-ATCC, respectively. The authors went one step beyond genome sequencing and performed a screening of a high-density transposon insertional mutant library with 16,512 clones against the host *Brassica oleracea*. This effort confirmed previous predictions of pathogenicity-related genes and revealed new ones, in particular, some were found in a chromosomal region specific of *Xanthomonas campestris* pv. *campestris*-8004 with respect to *Xanthomonas campestris* pv. *campestris*-ATCC. This shows a direct correlation between genome dynamics and *Xanthomonas campestris* pv. *campestris* virulence, an important result (Setubal et al. 2005).

13.4 Methods Involved Studying Diversity in Seed-Borne Pathogens

The arrival of molecular biology has caused a considerable change in the types of approaches used to identify and diversity analysis of seed-borne bacterial pathogens and to develop disease management strategies. This shift is driven by both technology and ecology and has occurred in parallel with significant changes in agricultural production methods, as highlighted by advances in precision agriculture and ecology-based pest management approaches. Where, precision agriculture has been driven primarily by technology and requires enormous information sets and a collection of information technologies to design crop-protection strategies. The

development of this essential knowledge has been advanced dramatically by the application of molecular genetical approaches to microbial ecology and has resulted in the birth of a new branch of research, termed molecular microbial ecology. Molecular methods to establish the nature and population dynamics of microorganisms form a major component of molecular microbial ecology and are also very useful to identify and characterize seed-borne pathogens (Louws et al. 1999).

Many protocols have been commonly employed to fingerprint phytobacteria/seed-borne pathogens, including arbitrarily primed or randomly amplified polymorphic DNA, repetitive sequence-based PCR (rep-PCR), and amplified fragment length polymorphism (AFLP) analysis. Each of these protocols generates a collection of genomic fragments via PCR, which are resolved as banding patterns that provide a high level of taxonomic resolution. Molecular techniques based on hybridization or amplification, and especially on polymerase chain reaction (PCR), have been developed for the most important plant pathogenic fungi, viruses, and bacteria. PCR has many beneficial characteristics that make it highly applicable for detecting seed-borne pathogens. These include speed (completed within 2–3 h), specificity (DNA probes can be designed to amplify nucleic acids from the desired genus, species, subspecies, race, etc.), sensitivity (single copies of nucleic acids can be detected after amplification), and easy objective result interpretation of genetic diversity (amplified sequence present in a DNA fragment of specific size indicates the presence of variation among pathogen). Because of this great potential, over the past 10 years, many PCR-based assays have been reported for seed-borne pathogens.

Polymerase chain reaction (PCR) is the in vitro, primer-directed, enzymatic amplification of nucleic acids (Saiki et al. 1988). This technique has been used in many diverse applications including diversity diagnosis of plant/seed-borne diseases. For PCR, primers designed to anneal to specific DNA sequences in the target organism's chromosomal DNA or RNA hybridize with and direct amplification of millions of copies of the target sequence. This amplified DNA can be visualized after electrophoresis in ethidium bromide-stained agarose gels. The remarkable proliferation of PCR-based methods for studying diversity of pathogens in seeds has provided very useful tools that are available and have begun to be implemented, in the vegetable seed industry and in some official seed testing laboratories for quality control testing (Agarwal 2006). PCR is considered to be too sensitive to be routinely applied as a tool to study genetic diversity and seed health assay. There were also concerns about the ability of PCR to distinguish between dead and viable cells. PCR is a sensitive technique and, theoretically, capable of detecting a single bacterial cell; the sample size and volume of seeds (e.g., 30,000 seeds/litre of buffer) being tested in conjunction with the small volume (~4 μl) that can be used as template in the PCR reaction, make PCR sensitive than many other techniques. As a consequence, other approaches, such as nested PCR, have been used to study detection and diversity of pathogens in seeds.

Likewise, technological advances in molecular biology have radically changed our capacity to rapidly characterize and track microorganisms. Complete genomic DNA sequences of microorganisms have provided an extensive

framework for a new era of microbial genetics, classification, and identification. These genomic approaches have also generated molecular tools for rapid and high-resolution pathogen detection and disease diagnosis. In fact, for novel or nonculturable pathogens, 16S rDNA sequence analysis has provided knowledge about the biology and ecology of the pathogen based on the nature of its "nearest neighbors" in the phylogenetic "tree of life". Nested PCR increases sensitivity by utilizing a second round of amplification using primers designed to anneal to internal regions of the amplicon produced by the first round of amplification. Using nested PCR, Poussier et al. (2002) detected *Ralstonia solanacearum*, the causal agent of bacterial wilt in tomato seeds. Nucleic acid-based methods tend to be relatively expensive to apply, and the advantages of these more rapid methods have to be considered against the less cost (but greater inconvenience to the grower, of the longer period required to achieve identification and diversity of a pathogen) of isolation-based methods.

Three Ds—diversity, detection, and diagnosis—provide a framework for the application of different PCR-based genome analysis protocols. "Diversity" refers to the degree of genetic variation within bacterial populations and relates to bacterial systematics, at multiple taxonomic or phylogenetic levels, as well as to the structure of pathogen populations (Louws et al. 1999). Our key premise is that assessing genetic diversity of populations is required to establish a stable taxonomy and developing durable disease management strategies for pathogenic bacteria. Such an approach is analogous to mapping soil variation that provides global position coordinates for precision agriculture; the latter cannot be accomplished without the former and the level of detail required is dependent on the objectives. For example, the genetic diversity "maps," most probably in the form of dendrograms or phylogenetic trees, can "provide orderly images of the natural structure of bacterial variation at many taxonomic levels". Thorough assessment of diversity will lead to better classification schemes. Once a stable classification has been established, well-grounded detection and plant disease diagnosis protocols can be devised, new isolates can be quickly characterized, problematic groups of strains can be proactively addressed, and specific ecological research questions can be more vigorously pursued (Fig. 13.4).

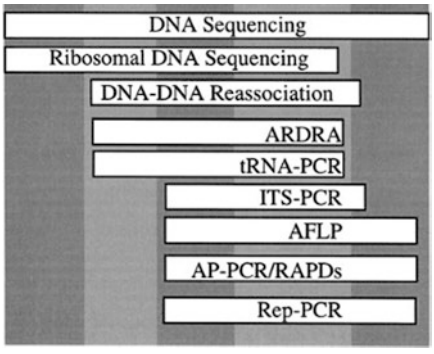

Fig. 13.4 Development of molecular methods for identification of seed-borne bacterial pathogens. Adapted from Louws et al. (1999)

13.4.1 Some Recent Genomic Fingerprinting Protocols

13.4.1.1 Low-Stringency Single Specific Primer-Polymerase Chain Reaction (LSSP-PCR)

Nucleic acid-based amplification from polymerase chain reaction (PCR) is a potential method for the rapid identification of a large number of plant pathogenic bacteria. Low-stringency single specific primer-PCR (LSSP-PCR) is a rapid and simple technique that detects sequence dissimilarity in DNA fragments by amplification in low-stringency conditions with a single specific primer for one of the extremities of the template (Pena et al. 1994). LSSP-PCR represents an important alternative PCR technique involving a simple repetition of the PCR process with one of the two primers used in the initial amplifications, but it is highly sensitive to the sequence content of the gene fragment being analyzed (Fig. 13.5). Furthermore, variations in the sequence of the amplified product can assist in the precise identification of the infecting organism to the species and pathovar levels. The individual genetic profiles of *R. solanacearum* isolates were obtained by LSSP-PCR from seed, soil, and plant material; the polymorphic variation discriminates pathogenic and non-pathogenic isolates by LSSP polymorphic banding patterns. The genetic variation of LSSP was confirmed by sequence analysis of isolates. The results correlated with the genetic variability of *R. solanacearum*, discriminated from diverse host (Prakasha et al. 2016). Even though the technique has great advantages toward

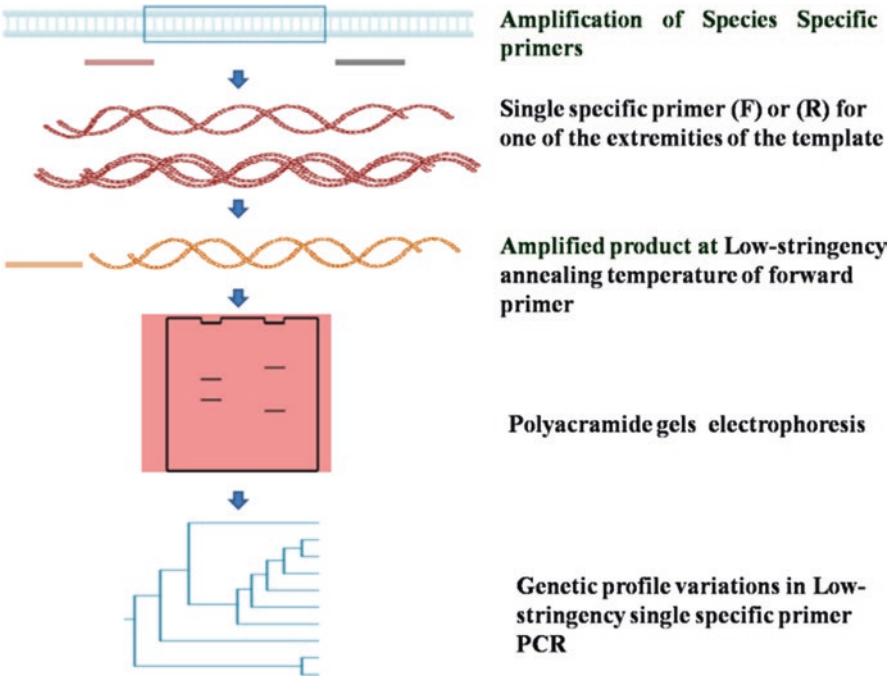

Fig. 13.5 Representation of low-stringency single specific primer-PCR (LSSP-PCR)

molecular detection and study of genetic variation, the technique was exploited less, and few reports were available in the area of LSSP-PCR.

13.4.1.2 Whole Genome Sequencing

In the last decade, the emergence of whole genome sequencing of prokaryotes has led to a wave of genomics-enabled research to dissect the genetics of bacterial phytopathogens and their interactions with host plants. This period has seen a remarkable transition from using Sanger sequencing to next-generation sequencing (NGS) technologies, which have facilitated the sequencing, assembly, and annotation of the entire hereditary blueprint of a prokaryote in less than 24 h. The most recent advances in NGS are examined, illustrating both the benefits and drawbacks of taking such a "brute force" approach. Existing techniques are also described for managing the unprecedented amount of data generated by NGS, for the successful assembly, annotation, and visualization of a bacterial genome.

The sequencing of prokaryote genomes started in earnest in 1995, with the publication of the DNA sequences of *Haemophilus influenzae* and *Mycoplasma genitalium* (Fraser et al. 1995). It wasn't, however, until the completion of the genome sequence of *Xylella fastidiosa*, the causative agent of citrus variegated chlorosis, that the genomics era finally reached plant pathology. The last decade has seen a proliferation in the sequencing of bacterial genomes. In 2009 the 1000th prokaryote genome sequence was completed (Lagesen et al. 2010). Although plant pathogenic bacteria are under-represented among these genome sequences, for *Pseudomonas syringae* alone, with the emergence of bacterial genomics, the entire hereditary blueprint of an organism can be acquired.

Genome-sequencing efforts have primarily involved culturable organisms because high-purity DNA preparations from a single isolate can be obtained. With the advent of improved sequencing technologies, coupled with increased throughput and decreased costs, metagenomics, in which a population of organisms is sequenced and can be employed. The genome sequence of *Candidatus* was obtained using metagenomics by whole genome shotgun sequencing of an infected psyllid, thereby bypassing the need for culturing of the pathogen. Analysis of the *Candidatus* genome revealed a highly reduced genome that is consistent with its lifestyle as an intracellular pathogen and insect symbiont. Intriguingly, analysis of the gene complement involving biochemical processes revealed auxotrophy for five amino acids that could improve efforts to culture this organism.

The diversity and breadth of organisms that can cause diseases on plants are vast. To facilitate access to the growing body of plant pathogen genome sequence, the Comprehensive Phytopathogen Genomics Resource (CPGR) has been created that serves as a portal to all publicly available plant pathogen genome sequence data and projects. Establishment of the CPGR Warehouse with accompanying metadata provides a broad, yet detailed, view of the status of plant pathogen genome sequence data. Not only complete, publicly available data sets are available, but planned and in-progress projects are collated. The CPGR supports researchers to quickly assess and obtain the genome sequence for their organisms of interest obviating the need to have either personal knowledge of the status of genomics initiatives or having to

search in multiple locations for information. In addition to the Warehouse, the CPGR offers display, search, and access tools to the genome sequence and annotation of 74 genomes and 82 transcriptomes. In addition, rDNA sequences are provided for 17,613 species to facilitate diagnostic marker development (Hamilton et al. 2011).

Several examples show the power of genomics to advance basic research; genomics can and will have a significant role in deciphering pathogen population structure and its relationship to disease, as well as in the development of diagnostic markers for seed-borne bacterial pathogens. For example, a number of detection methods rely on DNA-based markers where a targeted locus (loci) is amplified from the pathogen using PCR or detected through hybridization. Typically, these DNA-based markers can be scored in a binary fashion (present/absent), by size polymorphism, or by the kinetic nature of the amplification reaction (real-time PCR). Perhaps the most challenging aspect of developing a DNA-based marker for diagnostics is identifying unique or distinguishing loci within the target organism to provide a high resolution of detection, perhaps at the pathovar or race level. The usefulness of the CPGR as a resource was validated in the development of highly specific PCR-based diagnostic markers that distinguished *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola*, the causal agents of bacterial blight and bacterial leaf streak of rice, respectively. These pathovars, which are on the USDA-APHIS Select Agent list (http://www.aphis.usda.gov/programs/ag_selectagent/agbioterr_toxinlist.shtml), cannot easily be differentiated by morphological or physiological characteristics in culture. Using the comparative and computational resources within the CPGR, sets of unique and conserved loci were identified. These lists of candidate markers were then screened in a panel of *Xanthomonas* strains using PCR to validate the bioinformatics prediction of their phylogenetic distribution. Due to the availability of genome sequences from not only the target species (*X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*) but also other species of *Xanthomonas*, delineation of bona fide markers from the candidate list was straightforward, demonstrating the power of genomics, coupled with bioinformatics, to facilitate diagnostic marker development (Hamilton et al. 2011).

13.5 Applications of Genetic Diversity Assessment Methods

DNA fingerprinting protocols and their relationship to bacterial taxonomy have been studied with regard to seed-borne bacteria; studies have been described to map the diversity of known species within a complex genus such as *Xanthomonas*, *Pseudomonas*, and *Clavibacter*. For the careful analysis of how rep-PCR genomic fingerprint protocols compare with other techniques for clustering strains and assessing diversity focus is on *Xanthomonas* as a model system. This genus is of major economic importance worldwide and currently comprises 20 DNA homology groups (species), determined using a polyphasic approach, including data from the largest matrix of DNA-DNA homology values currently available for any bacterial genus. Genomic fingerprints generated from over 350 *Xanthomonas* strains with

BOX, ERIC, and REP primers were linearly combined into one data set using GelCompar software. Cluster analysis of the combined genomic fingerprint profiles, and to a somewhat lesser extent the separate profiles, grouped strains according to their species designation (DNA homology group), with the exception of the more heterologous DNA homology groups, which comprises several genomic subgroups. Therefore, the rep-PCR genomic fingerprinting analysis confirmed the DNA-DNA homology-based classification scheme and provided additional data concerning genomic homogeneity or diversity at the subspecies level, refining current concepts of *Xanthomonas* taxonomy.

In a detailed study of 179 strains characterized by rep-PCR and 82 strains by AFLP analysis, the similarity values generated by the DNA fingerprinting protocols were compared with the corresponding DNA-DNA homology values (n = 732 and 294, respectively). The similarities between genomic fingerprints were found to directly reflect DNA-DNA hybridization content similarities among strains within the genus. This high level of congruence between DNA fingerprint profiles and DNA-DNA homology values suggests that rapid genomic fingerprinting techniques may preclude, complement, or even replace DNA-DNA homology experiments to clearly define *Xanthomonas* and other bacterial species under the present nomenclature scheme. This is especially important for studies directed to characterize large collections of culturable but otherwise uncharacterized environmental microorganisms. Parallel studies using clinical bacterial isolates have come to similar conclusions, which confirm the general applicability of genomic fingerprinting analyses for species definition.

PCR-based genomic fingerprinting protocols have proven effective in assessing diversity of strains within a host-adapted population. Bacterial populations are notoriously "shifty enemies", and through mutation, recombination, and migration, complemented with random drift and selection pressure, they often circumvent disease management strategies, such as the use of bactericides and resistant cultivars. Although the evolution of novel strains and dynamics of populations cannot be predicted, the development of disease management strategies, such as host resistance deployment and specific diagnostic protocols, can be advanced based on knowledge of the population structure. Studies of population structure seek to determine the amount of genetic variation and phylogenetic relationships among individuals or subpopulations within a bacterial population. Such studies must include a careful analysis of the temporal, spatial, and pathogenic variation of the pathogen, a very laborious task when using protocols such as RFLP but feasible using PCR-based genomic fingerprinting protocols.

RAPD analysis of *X. hortorum* pv. *pelargonii*, *X. fragariae*, *E. amylovora*, and *Xylella fastidiosa* populations revealed limited genetic variability. In contrast, the population of strains able to cause bacterial spot of tomato has been found to be comprised of genetically very different organisms that have completely different rep-PCR genomic fingerprints. In fact, the two predominant groups are different species classified as *X. axonopodis* pv. *vesicatoria* and *X. vesicatoria*. These two species incite virtually the same disease, but their biochemical characteristics and virulence levels differ substantially, and genetic resistance developed against *X.*

axonopodis pv. *vesicatoria* is not effective against *X. vesicatoria*. Rep-PCR genomic fingerprinting was used to map the genetic diversity of *X. axonopodis* pv. *vesicatoria* strains isolated in the Caribbean and Central America. In many countries or islands, *X. axonopodis* pv. *vesicatoria* was found to be the predominant group, and *X. vesicatoria* was absent. Island-specific lineages were also found, which suggests that migration events were rare. The absence of *X. vesicatoria* strains and evidence that migration may be of limited importance provide an informed basis to deploy host resistance to *X. axonopodis* pv. *vesicatoria* strains with confidence (Hamilton et al. 2011).

Deployment of rice varieties has been subjected to boom-and-bust cycles, and effective resistance to the bacterial blight pathogen, *X. oryzae* pv. *oryzae*, is often short-lived, owing to a shift in the pathogen population toward virulent races. The population of *X. oryzae* pv. *oryzae* is comprised of genomic lineages that can be readily discerned based on rep-PCR genomic fingerprint polymorphisms. Rep-PCR genomic fingerprinting analysis combined with pathotype analysis provided improved insight about genetic diversity at a microgeographic scale and among countries, again confirming the utility of genomic fingerprinting as an effective tool to better understand the dynamics and evolution of bacterial populations. ERIC-PCR genomic fingerprinting has discerned host-adapted clusters of strains within the heterologous *Pseudomonas syringae* pv. *syringae* group. RAPD and AFLP analyses generated 27 unique patterns among 30 closely related pseudomonads. AFLP was effective in discerning genetic diversity within and among pathovars of *X. translucens* and supported conclusions that pathovars *cerealis*, *translucens*, and *undulosa* are true biological entities.

Next-generation sequencing methodologies, in which ultra-high-throughput sequencing capabilities are coupled with highly reduced costs, enable new research directions due to the inherent paradigm-changing scale of data generation. Certainly, data handling and mining will be a large challenge and a bottleneck that needs to be addressed. However, bioinformatics solutions such as Galaxy, an open-source platform for next-generation sequencing computational efforts, are emerging to handle and process these data sets. The Comprehensive Phytopathogen Genomics Resource (CPGR) is already incorporating data from these methodologies and merging them with data generated from "first-generation sequencing platforms". Assembled genomes sequenced with next-generation sequencing technologies can be readily incorporated into the A Systematic Annotation Package (ASAP) and CPGR databases. In fact, 17 of the genomes obtained from ASAP were generated using next-generation sequencing technologies and were seamlessly incorporated into the CPGR. Other amenable data sets include RNA-seq data sets in which mRNA is converted into cDNA and sequenced using short read next-generation platforms. Algorithms are available to perform de novo assemblies of these transcriptomes that can be readily incorporated into the CPGR Transcript Assemblies database. Whereas the CPGR can currently handle the volume of plant pathogen genomes being deposited in NCBI, the pace at which genomes are being generated along with the large range in quality of genome and transcriptomes generated will become prohibitive. As a consequence, standards for the quality of the underlying sequence for inclusion

in the CPGR will need to be invoked. As these next-generation sequencing methods improve, quality criteria for reads, assemblies, and annotations will stabilize and permit community-defined quality standards for genome projects that can be applied to target genomes for the CPGR. Clearly, there is enormous potential for genomic data to shape biology, including plant pathology, and the CPGR provides a portal for plant pathologists to determine the genome sequence status of their organism of interest, data mine these bacterial and eukaryotic genomes, and identify candidate markers for diagnostic marker development (Jimenez-Lopez et al. 2013).

13.6 Conclusion

On the one hand, global population is exploding in the geometric progression, whereas food production is increasing in the arithmetic progression. Seeds are the primary input of agriculture, on which all other secondary investments depend. Seed quality plays a pivotal role in the agricultural production. Seeds growing in agricultural field or in natural circumstances are exposed to numerous dissimilar species of pathogen. Seed-borne pathogens are a continuing problem and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas. The arrival of molecular biology has caused a considerable change in the types of approaches used to identify and diversity analysis of seed-borne bacterial pathogens and to develop disease management strategies. Nucleic acid-based amplification using polymerase chain reaction (PCR) is a potential method for the rapid identification of a large number of plant pathogenic bacteria. PCR-based diversity studies are generally accomplished with universal primers that generate an array of DNA amplicons frequently referred to as genomic fingerprints. Next-generation sequencing methodologies, in which ultra-high-throughput sequencing capabilities are coupled with highly reduced costs, enable new research directions due to the inherent paradigm-changing scale of data generation.

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Genetic Diversity, Transmission and Understanding of Seed-Borne Viruses and Phytoplasma

14

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Abstract

Seed-borne plant viruses and phytoplasma are the major constraints in reducing the yield of many agricultural and horticultural crops worldwide. In this chapter, we have discussed about the genetic diversity, transmission and distribution of some plant viruses and phytoplasma originating from different geographical locations. At present, more than 200 plant viruses, phytoplasma and viroids are known to be seed-transmitted of which most of them were reported from crops belonging to Leguminosae family. Seed-transmitted viruses were reported from various genera including Poty-, Tospo-, Begomo-, Nepo-, Crypto-, Ilar-, Tobamo-, Potex-, Como-, Carla-, Carmo-, Cucumo-, Sobemo-, Furo-, Bromo- and Tymoviruses. Besides seed-transmission, more than 100 viruses are known to be transmitted through vegetative planting materials in different crops. Although the rate of infection due to seed-borne viruses is less as compared with those viruses transmitted by different insect vectors, the seed-borne viruses cause enormous quality and economic losses in various crops ranging from 4 to 100%. To manage these seed-borne viral diseases, understanding about their spread and population structure of viruses is very much required. In view of this, certain seed-borne viruses including *Cucumber mosaic virus* (CMV), *Pea seed-borne mosaic virus* (PSbMV), *Soybean mosaic virus* (SbMV), *Zucchini yellow mosaic virus* (ZYMV) and some viruses propagated through vegetative cuttings including

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Sugarcane mosaic virus (SCMV) and *Sugarcane yellow leaf virus* (SCYLV) have been discussed in this chapter.

14.1 Introduction

World is having enough capacity to produce sufficient food to feed the growing population. Besides the improvement in agricultural scenario since last two decades, 793 million people are still facing the problem of hunger. According to FAO (2013), it has been estimated that 161 million malnourished includes the children below 5 years of age. Worldwide, 'Hidden Hunger' or micronutrient deficiencies affect more than two billion people, resulting in poor socio-economic and physical development. The world hunger forced policy-makers and leaders to think and to carry out various high-level programmes and to design strategies to cope up with the hunger, food insecurity and malnutrition. More recently, during Rio+20 summit organized by United Nations held at Rio de Janeiro, 'Zero Hunger Challenge' programme for formation of new policies and their implementation against hunger was initiated.

Hunger and food insecurity are the two sides of poverty. According to FAO, today's world population is around 6.5 billion and by 2050 it will be around 9 billion. The challenges in world agricultural scenario are to reduce the hunger and food insecurity, and this can be achieved very effectively by growth in agriculture sector. In order to mitigate the challenges, improvement in productivity, quality and profitability are the key elements to be concentrated upon. To enhance productivity, there are several constraints including biotic and abiotic stresses and climatic changes. Among the biotic constraints, viral diseases are one of the major limitations in agricultural and horticultural production worldwide. In food grains, legumes, vegetative propagated and other crops, the seed-borne viruses are one of the limitations in enhancing crop/seed production.

Till date, there are more than 200 plant viruses, viroids and phytoplasma reported to be seed-transmitted in different crop plants. Moreover, more than 60 viruses infecting leguminous crops are known to be seed-transmitted (King et al. 2012). Seed-transmitted viruses belong to different genera viz., Alfamo-, Begomo-, Bromo-, Capill-, Carla-, Carmo-, Caulimo-, Como-, Crypto-, Cucumo-, Enamo-, Faba-, Furo-, Hordei-, Ilar-, Necro-, Nepo-, Potex-, Poty-, Sobemo-, Tobamo-, Tobra-, Tombus-, Tospo- and Tymoviruses. Largest number of seed-transmitted viruses were recorded from Potyvirus group (35) followed by Nepo-(28), Crypto-(28), Ilar-(14), Tobamo-(7), Potex-(7), Como-(6), Carla-(5), Carmo-(5), Cucumo-(5), Sobemo-(5), Furo-(4), Bromo-(3) Tymo (3), Begomo-(3) and Orthotospo-(1) virus groups. Whereas, very few were recorded as seed-transmitted from the rest of the virus groups (King et al. 2012; Maes et al. 2018). Recently, in soybean seed transmission of *Soybean vein necrosis virus* (SVNV; Groves et al. 2016) and *Tomato yellow leaf curl virus* (TYLCV; Kil et al. 2017) have been reported from USA and Korea, respectively.

Besides legume crops, there are some economically important viruses which are propagated through vegetative planting materials like stem cutting, rhizomes, setts, suckers, tubers, corms, bulbs, etc. Worldwide, different viruses, viroids and phytoplasma are known to be transmitted through vegetative planting materials in different crops including sugarcane, banana, potato, sweet potato, black gram, citrus, cassava, pineapple, onion, garlic, chrysanthemum and many other crops (Sastry 2013; Kim et al. 2015; Kothandaraman et al. 2016).

To manage these pathogens, very effective strategy has been the use of resistant cultivars. This requires a complete understanding of the genetic diversity of the pathogens as well as the interaction with cultivar, because resistance breakdown can occur when new strains of viruses appear (Grisham and Pan 2007). In addition, careful planning of crop management practices, including time of planting and harvesting, are used for disease control. Recently genetically engineered transgenic resistance has been used in attempts to control plant virus diseases (Beachy 1997; Gonsalves 1998). Despite the numerous potential benefits offered by the use of transgenic plants for virus disease control, concerns have been raised regarding their widespread use. One concern has been the possible emergence of new viruses or virus genotypes that could overcome the engineered resistance and subsequently affect virus and host plant ecology (Tepfer 1993; Robinson 1996; Aaziz and Tepfer 1999). This may be due to occurrence of recombination between an infecting mutant virus and transgenic plants expressing wild-type genes of the same virus (Greene and Allison 1994; Allison et al. 1996; Wintermantel and Schoelz 1996). Secondly, it is possible that virus genotypes that are able to infect transgenically derived resistant plants may already exist in natural various populations and they could gradually take advantage of the niches available after the deployment of resistant plants. Thirdly, RNA viruses have potential for high genetic variation due to absence of RNA replicase proofreading ability.

Further natural populations of RNA viruses exhibit extremely high levels of genetic diversity because of high mutation rates, rapid replication rates and large population sizes (Duffy et al. 2008). Understanding the patterns of intra-host viral diversity is the key to understand the underlying evolutionary mechanisms in RNA viruses, as high levels of genetic diversity have been linked to the capacity of these viruses to evade host resistance mechanisms (Lech et al. 1996; Feuer et al. 1999), to adopt to new niches (Roossinck 1997), switch hosts (Jerzak et al. 2008) and alter virulence (Acosta et al. 2011).

The genetic variability of viruses affects their detection by serological and molecular methods and also their control when host resistance is deployed. Knowledge of intra-specific viral diversity is required to determine whether the virus-specific antibodies, polymerase chain reaction (PCR) primers or nucleic acid probes available are likely to detect different virus isolates of diverse geographical origin. Similarly, many natural and engineered mechanisms of virus resistance are strain-specific which makes them prone to be overcome.

Some studies showed that there are at least three bottlenecks which were thought to restrict the genetic diversity in plant viral populations: (1) vector acquisition/transmission, (2) systemic movement within the host plant and (3) during seed

(vertical) transmission. For instance, inter plant transmission results in reduction of genetic variation and it was estimated that number of virions transmitted per event by aphids to be in the order of 1–3 for *Potato virus Y* and *Cucumber mosaic virus* (Moury et al. 2007; Betancourt et al. 2008). But currently there is no data on the extent and consequences of the genetic bottlenecks associated with seed transmission. Seed transmission is an important mechanism by which viruses persist between crop seasons, and that even a seed transmission rate as low as 0.001% can initiate an epidemic (Ryder 1973). Understanding how seed transmission shapes viral genetic diversity is fundamental to control the seed-borne viral pathogens. However, the success of seed transmission is determined by temporal and spatial shifts in virus population, because virus infects the seed prior to causing infection in the subsequent seedling. Studies on ZYMV suggest that significant genetic diversity is transmitted via seeds. Hence, vertically infected seedlings may be instrumental in dissemination of viral pathogens throughout the worldwide.

A single virus isolate does not consist of a single RNA sequence, but of a population of related sequence variants, often referred to as quasi-species (Holland et al. 1992; Domingo et al. 1995; Eigen 1996). The quasi-species nature of RNA viruses implies a high adaptive potential, allowing for the rapid selection of biologically distinct sequence variants with the highest fitness in new environments. Thus, if present, sequence variant(s) capable of overcoming transgenic resistance could rapidly come to dominate the virus population allowing for a resistance-breaking phenotype. Therefore, knowledge of virus population diversity relative to transgenic (and even conventional) resistance is needed. In this chapter the recent information on the genetic diversity and transmission of *Cucumber mosaic virus* (CMV), *Pea seed-borne mosaic virus* (PSbMV), *Soybean mosaic virus* (SbMV), *Sugarcane mosaic virus* (SCMV), *Sugarcane yellow leaf virus* (SCYLV) and *Zucchini yellow mosaic virus* (ZYMV) have been discussed in detail. Moreover, the distribution of seed-transmitted viruses in some oilseeds and pulses are listed (Table 14.1).

14.2 Economic Impact

Plant viruses cause enormous crop losses in different agricultural or horticultural crops. The entire crop loss due to plant viruses is determined based on the stage of viral infection, severity of infection, reaction of the hosts and conducive environment for its multiplication in the system. Estimation of the crop loss has to be monitored under controlled conditions by mechanical transmission of the virus along with the healthy control and under natural conditions (Ruesink and Irwin 2006). However, it is difficult to exactly pin-point crop loss by single viral pathogen under field conditions due to association of several other biotic and abiotic factors. In order to minimize the crop loss, there is a need to avoid early or seed-borne infections. There are many reports on seed-transmitted viruses causing economic losses to different crops worldwide (Table 14.2).

Table 14.1 Distribution of seed-transmitted viral diseases of different crops worldwide with their causal virus, genus and family

Crop	Disease	Causal virus (acronym)	Genus (family)	Country	References
Legumes					
Soybean	Bud blight	*Tobacco ringspot virus* (TRSV)	*Nepovirus*	USA, China and India	Hill (2003) and Frison et al. (1990)
	Dwarf	*Soybean dwarf virus* (SbDV)	*Luteovirus*	Japan, Indonesia, Africa, Australia and New Zealand	Hartman et al. (1999)
	Vein necrosis	*Tomato spotted wilt virus* (TSWV)	*Orthtospovirus*	USA	Groves et al. (2016)
	Leaf curl	*Tomato yellow leaf curl virus* (TYLCV)	*Begomovirus*	Korea	Kil et al. (2017)
Groundnut	Stem necrosis	*Tobacco streak virus* (TSV)	*Ilarvirus*	India	Reddy et al. (2001)
	Clump	*Peanut clump virus* (PCV)	*Pecluvirus*	Pakistan and West Africa	Bragard et al. (2008)
		Indian peanut clump virus (IPCV)		India	Reddy et al. (2008)
	Mottle	*Peanut mottle virus* (PeMoV)	*Potyvirus*	China, Georgia and India	Reddy and Thirumala Devi (2003)
		Peanut stripe virus (PStV)			
	Stripe			China and Georgia	Lynch et al. (1988) and McDonald et al. (1998)
	Yellow mosaic	*Cucumber mosaic virus* (CMV)	*Cucumovirus*	China and Argentina	Xu and Barnett (1984)
Common bean	Common mosaic and black root	*Bean common mosaic virus* (BCMV)	*Potyvirus*	Europe, India, Peru and Spain	Morales (2003), Sharma et al. (2008), and Ferreira et al. (2012)
		Bean common mosaic necrosis virus (BCMNV)		Eastern Africa, Dominican Republic Haiti and Tanzania	Morales (2003) and Chilangane et al. (2013)
	Mosaic	*Cucumber mosaic virus* (CMV)	*Cucumovirus*	Chiley, Iran and Turkey	Morales (2003) and Schwartz et al. (2005)

(continued)

Table 14.1 (continued)

Crop	Disease	Causal virus (acronym)	Genus (family)	Country	References
Cowpea	Mosaic	*Cowpea aphid-borne mosaic virus* (CABMV) and *Bean common mosaic virus*- Blackeye cowpea mosaic strain (BCMV-BICM)	*Potyvirus*	Ghana, West Africa, Nigeria and Burkina Faso	Bashir and Hampton (1996) and Salem et al. (2010)
	Mosaic	*Cowpea mosaic virus* (CPMV) and *Cowpea severe mosaic virus* (CPSMV)	*Comovirus*	Most of the cowpea producing countries	Bashir and Hampton (1996) and Salem et al. (2010)
	Mosaic	*Southern bean mosaic virus* (SBMV)	*Sobemovirus*		
	Stunt	*Cucumber mosaic virus* (CMV) and *Bean common mosaic virus*- Blackeye Cowpea mosaic strain (BCMV-BICM)			
	Mild mottle	*Cowpea mild mottle virus* (CMMV)	*Carlavirus*		
		Cowpea mottle virus (CPMoV)	*Carmovirus*		
Pea	Mosaic	*Pea-seed-borne mosaic virus* (PSbMV)	*Potyvirus*	Czech Republic	Kraft and Pfleger (2001)
		Bean yellow mosaic virus (BYMV)		Syria, Egypt, Italy and Libya	Kraft and Pfleger (2001) and Makkouk et al. (2012)

(continued)

Table 14.1 (continued)

Crop	Disease	Causal virus (acronym)	Genus (family)	Country	References
Faba bean	Mosaic and necrosis	*Bean yellow mosaic virus* (BYMV)	*Potyvirus*	Egypt, Iraq, Sudan, Israel, Italy, Lebanon, Libya, Morocco, Syria, Tunisia, Greece and Turkey	Makkouk et al. (2012) and El-Bramawy and El-Beshehy (2012)
	Mottle	*Broad bean mottle virus* (BBMV)	*Bromovirus*	Morocco, Sudan, Tunisia, Syria, Egypt and Algeria	Makkouk et al. (2012)
Lentil	Mosaic and mottle	*Pea-seed-borne mosaic virus* (PSbMV)	*Potyvirus*	Algeria, Egypt, Ethiopia, Iran, Iraq, Jordan, Morocco, New Zealand, Pakistan, Syria, Tunisia and Turkey	Kumari et al. (2009)
		Cucumber mosaic virus (CMV)	*Cucumovirus*	Australia, Ethiopia, India, Iran, Nepal, New Zealand, Pakistan and Syria	Kumari et al. (2009)

14.3 Genome Organization and Genetic Diversity

14.3.1 *Cucumber Mosaic Virus* (CMV)

Cucumber mosaic virus (CMV), the type member of the genus *Cucumovirus* in the family *Bromoviridae*, is one of the most economically important widespread viruses with a host range including plants from approximately 365 genera and at least 85 families (Palukaitis et al. 1992). The CMV genome consists of three positive-sense single-stranded RNAs and two sub-genomic RNAs (Ding et al. 1994). RNA1 encodes 1a protein possessing methyltransferase and helicase domains (Guan et al. 2004) whereas RNA2 encodes two proteins 2a and 2b. The 2a protein encodes RNA-dependent-RNA polymerase (RdRp) and forms the replicase complex together with the 1a protein (Habili and Symons 1989; Hayes and Buck 1990). The 2b protein, a multifunctional protein functions in host-specific, long-distance movement, symptom induction and as a virulence determinant by suppressing gene silencing (Diaz et al. 2007). Recent studies showed that 2b gene also plays an important role in the selection of inter viral recombination (Shi et al. 2008). The CMV RNA3

Table 14.2 Crop losses caused by some seed-transmitted viruses in different crops worldwide

Crop	Causal virus	Crop loss (%)	Country	References
Lettuce	*Lettuce mosaic virus* (LMV)	4–100	USA	Broadbent et al. (1951), Grogan et al. (1952), and Zink et al. (1956)
Bean	*Bean common mosaic virus* (BCMV)	50–68	USA	Lockhart and Fischer (1974) and Hampton (1975)
Soybean	*Soybean mosaic virus* (SMV)	35	USA	Ross (1977)
		8–25	USA	Ross (1969)
Peanut	*Peanut mottle virus* (PMV)	31–47		Kuhn et al. (1978)
	Peanut stripe virus (PStV)	21–23	USA	Demski et al. (1984)
Cowpea	*Cowpea mosaic virus* (CpMV)	13–87		Kaiser and Mossahebi (1975) and Suarez and Gonzalez (1983)
	Cowpea banding mosaic virus (CpBMV)	11.5–43.5		Sharma and Varma (1981)
Barley	*Barley stripe mosaic virus* (BSMV)	35–40	USA	Eslick (1953)
Lentil	*Pea seed-borne mosaic virus* (PSbMV)	96	Western Australia	Coutts et al. (2008)
Potato	*Potato Virus* Y and *Potato leaf roll virus* (PLRV)	60–80	India	Garg (2005)
	Potato Virus X, *Potato Virus S* and *Potato Virus M*	10–30		
Cassava	*Cassava mosaic virus* (CMV)	11	Africa	Padwick (1956)
		20–95		Beck and Chant (1958), Terry and Hahn (1980), and Thresh et al. (1994)
Citrus	*Citrus tristeza virus* (CTV)	Up to 75	Brazil, Argentina, Israel, India, Uruguay and USA	Bennet and Costa (1949), Nolla and Fernandez (1976), and Klotz (1961)
Cocoa	*Cocoa swollen shoot virus* (CSSV)	25–50	Ghana	Crowdy and Posnette (1947)
Sugarcane	*Sugarcane yellow leaf virus* (SCYLV) and phytoplasma causing yellow leaf disease	50	Brazil	Marcone (2002)
		15	USA	

encodes two proteins, 3a a cell-to-cell movement protein (MP) and 3b a capsid protein (CP), translates from a sub-genomic RNA4 and plays a role in cell-to-cell movement, virus assembly and aphid-mediated transmission (Perry et al. 1994; Canto et al. 1997). Studies on host range, symptomatology, immunology and molecular analysis of the CMV genomic RNAs demonstrate that isolates of CMV are heterogeneous (Palukaitis and Arenal 2003).

CMV isolates were classified into two main subgroups designated as subgroup I and II based on serology (Palukaitis et al. 1992; Wahyuni et al. 1992; Ilardi et al. 1995), nucleic acid hybridization (Owen and Palukaitis 1988), RT-PCR followed by RFLP (Rizos et al. 1992) and nucleotide sequence identity (Palukaitis et al. 1992; Roossinck 2002). However, based on sequence similarity and phylogenetic relationship CMV subgroup I was further subdivided into IA and IB possessing 75% nucleotide identity (Palukaitis and Zaitlin 1997; Roossinck 2002). Isolates of subgroup II demonstrate 96% identity, indicating that subgroup I is more heterogeneous than subgroup II (Palukaitis and Arenal 2003). Analysis of the CP gene and 5′untranslated region (UTR) of the RNA3 has led to further subdivision of subgroup I into subgroups IA and IB, with 92–95% nucleotide identity (Roossinck et al. 1999; Roossinck 2002). However, phylogenetic analysis of nucleotide sequences of some CMV strains showed that the estimated trees for various ORFs located on the different RNAs were not congruent and did not completely support the sub-grouping indicated by the CP ORF. This indicates that different RNAs may have independent evolutionary histories and re-assortment played an important role in CMV evolution (Roossinck 2002).

Further these subgroups are not evenly distributed across the agricultural regions. Subgroups IA and II have a worldwide distribution, while the majority of subgroup IB isolates are restricted to East Asia (Roossinck 2002; Moury 2004). However, CMV subgroup IB isolates have been reported from Italy and India (Gallitelli 2000; Bhat et al. 2004; Madhubala et al. 2005). Recently subgroup IB has been confirmed from both Hawaii and California (Roossinck et al. 1999; Lin et al. 2003).

14.3.2 *Pea Seed-Borne Mosaic Virus* (PSbMV)

Pea seed-borne mosaic virus (PSbMV) is a member of genus *Potyvirus* possessing flexuous, rod-shaped particles with positive sense ssRNA genome of approximately 10 kb in length (Hollings and Brunt 1981). PSbMV infected pea seeds acts as the main initial source of inoculum reservoir followed by aphid vectors for transmitting the virus from germinated seedlings to healthy pea plants. The PSbMV mainly uses the suspensor as a mode of entry into the embryonic tissues of pea. The suspensor during the early stages of seed development maintains close contact with the micropyle as well as endosperm wall (Wang and Maule 1994). PSbMV moves from the maternal cells to the embryo through micropyle, endoplasmic cytoplasm and embryonic suspensor (Roberts et al. 2003).

The first detailed evidence for the existence of PSbMV strains was demonstrated by Hampton et al. (1981). They compared seven PSbMV isolates, viz. PsS_2 from

Czechoslovakia (Musil 1970), WA-1 and WI-1 isolates from Washington (Stevenson and Hagedorn 1969; Knesek et al. 1974), p-202 isolate from Japan (Inouye 1967), a deviant type E. 224 of isolate E. 210 from the Netherlands (Bos 1970) and isolate C-4-24 from Oregon (Hampton et al. 1974). The seven isolates were characterized based on particle length, serological relationship and symptom development on 19 different pea lines. Later three more pathotypes of PSbMV viz., P-1, P-4 and L-1 have been identified on the basis of pathotype specific pea (*Pisum sativum*) and lentil (*Lens culinaris*) genotypes (Alconero et al. 1986). Interestingly, it was observed that the pathotype L-1 isolated from lentil was much more severe on pea cultivars. Recently, two other pathotypes U-1 and U-2 were reported from Pakistan which appear to represent additional genetic variants of the virus (Ali and Randles 1997).

14.3.3 *Soybean Mosaic Virus* (SMV)

Soybean mosaic virus (SMV), a member of the genus *Potyvirus* within the family *Potyviridae*, causes most widespread disease of soybean (Wang 2009). SMV has a single-stranded positive-sense RNA genome of 9.6 kb, packed in the coat protein subunits with viral genome-linked protein (VPg) at the 5′ end and a poly-A tail at the 3′ end in addition to the 5′ and 3′ non-translated regions (NTRs) (Reichmann et al. 1992; Adams et al. 2005; Gagarinova et al. 2008a). The SMV viral genome consists of a large open reading frame (ORF) and a small ORF that results from translational slippage in the large ORF (Jayaram et al. 1992; Chung et al. 2008). The two polyproteins encoded by these two ORFs are co- and post-translationally processed into 11 final products by 3 viral proteases (P1, HC-Pro and N1a-Pro). The 11 mature proteins beginning from the N terminus of the polyprotein are P1 (the first protein), HC-Pro (the helper component/protease), P3 (the third protein), P3N-PIPO (resulting from the frame-shift in the P3 cistron), 6K1 (the first 6 kDa peptide), CI (the cylindrical inclusion protein), 6K2 (the second 6 kDa peptide), NIa-VPg (nuclear inclusion a-viral genome-linked protein), NIa-Pro (nuclear inclusion a protein-the protease), NIb (nuclear inclusion b protein) and CP (coat protein) (Jayaram et al. 1992). Most of these viral proteins are multifunctional (Jayaram et al. 1992; Chung et al. 2008).

To date, a large number of SMV isolates have been reported in the world. SMV isolates have been classified into categories G1–G7 based on their ability to break resistance genes (Cho and Goodman 1979). Later, two more groups, G7A and C14, were added (Buzzell and Tu 1984; Lim 1985). Similarly, five (A to E) and eight (Sa to Sh) additional SMV strains have been reported in Japan and China, respectively (Takahashi et al. 1980; Chen et al. 1986). Recently, two other SMV isolates have been identified in Canada: a necrotic strain, SMV-N and a G2 isolate (Gagarinova et al. 2008a; Farsi et al. 2009; Wang 2009). But due to high sequence similarity of SMV-N and G2, SMV-N was considered as a G2 isolate (Gagarinova et al. 2008b).

SMV generally transmits from infected soybean maternal tissues to the next generation via seeds, although the mechanism remains unknown. Early infection of a

soybean plant can result in severe effects such as reduced pod set, seed size, poor oil content of the seed and root nodulation (Hill 1999). SMV infection can also lead to poor seed quality by causing reduced seedling viability, poor seedling vigor and seed coat mottling (El-Amretz et al. 1987; Hobbs et al. 2003). A study conducted on seed transmission of SMV isolates showed that the unmodified SMV G2 lacked seed transmission and the SMV 413 was seed-transmitted in 14% of the seedlings.

14.3.4 *Sugarcane Mosaic Virus* (SCMV)

In India, sugarcane mosaic disease was first reported on D 99 a sugarcane variety (introduced from USA) from Pusa, Bihar and on Sathi 131, an Indo-originated sugarcane variety of Bihar (Dastur 1923). Since then, this disease continued as one of the serious threat to sugarcane growers in India. This is transmitted from vegetative cuttings (Bhargava 1975; Rao et al. 1995). Sugarcane mosaic, one of the most important viral diseases of sugarcane, is widely distributed in the world and its economic significance varies among the regions (Koike and Gillaspie 1989). Economic losses due to sugarcane mosaic depend on varietal susceptibility, virus strain, its interaction with other diseases, vector population and environmental conditions (Goodman 1999). One of our study recorded the interveinal chlorotic specks and streaks on young leaves of sugarcane (Fig. 14.1a–c; Holkar et al. 2017) and detected the association of *Sugarcane mosaic virus* by RT-PCR. The RT-PCR products were cloned and sequenced. The amino acid sequence homology revealed the association of SCMV (Holkar et al. 2017). During 2002–2003, Hema et al. (2003) recorded another causal virus of mosaic disease which was named as *Sugarcane streak mosaic virus* (SCSMV). Since its first report, SCSMV was systematically studied in India and found that this virus was more widely associated with the mosaic disease (Rao et al. 2006; Vishwanathan et al. 2008a). Moreover, there were certain reports of mixed infections of these two viruses and caused significant yield losses in most of the cane varieties across the tropics and subtropics (Singh 2001; Hema et al. 2003; Viswanathan and Balamuralikrishnan, 2005; Viswanathan et al. 2007; Singh et al. 2009). The SCMV belongs to the *Potyvirus* genus and family *Potyviridae*, which cause yield losses of about 10–90% in India (Rao et al. 1998a, b; Singh 2001; Viswanathan and Balamuralikrishnan 2005). Whereas, SCSMV belongs to the undescribed new genus-*Susmovirus* (Hema et al. 2003; Adams et al. 2005). Vishwanathan et al. (2008a) studied the genetic diversity of Indian isolates (Coimbatore-CB) of *Sugarcane streak mosaic virus* (SCSMV), and among the SCSMV-CB isolates, 85.7–100% (nt) and 89.9–100% (aa) sequence identities were observed. Based on the phylogeny, the grouping of the isolates from India and USA was appeared in 16 different phylogenetic groups. A total of 52 SCSMV-CB isolates were distributed in 14 phylogenetic groups.

In China during 2008, the diversity of SCMV has been studied, wherein obtained 33 SCMV CP nucleotide sequences were 76.4–96.9% identical to the SCMV strains A, B, D, E and SC, and 76.7–99.8% were identical to each other. This suggested that the 33 isolates were identified as SCMV species according to the species

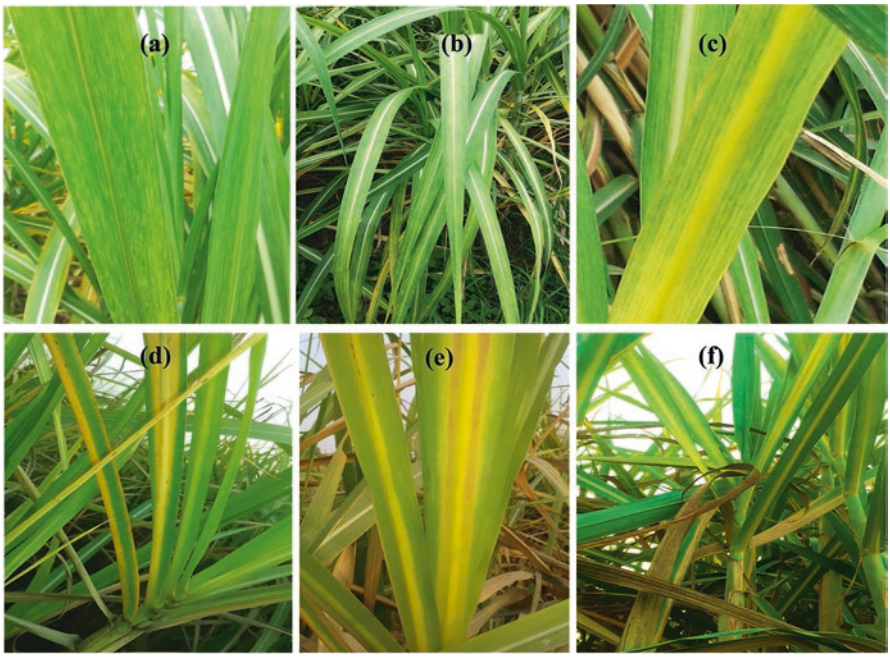

Fig. 14.1 Symptoms of some seed-transmitted diseases of sugarcane including sugarcane mosaic and yellow leaf diseases under field conditions. Mosaic affected sugarcane plants exhibiting shades of yellow and green patterns on lower leaf surface (**a**), on the upper leaf surface (**b**), and prominent streaks on both the leaf surfaces (**c**), yellow leaf disease affected plants showing smaller leaves and bunching at the crown region (**d**), yellowing of midribs (**e**) and severe yellowing of midribs with necrosis from tip to downward (**f**)

demarcation criteria of 76% nt identity, as described by Adams et al. (2005). The 206 SCMV isolates were divided into five different groups and two unique strains by phylogenetic tree. The first group, including the sugarcane or SCE group, was given by Alegria et al. (2003), which included 118 isolates, of which 104 were infecting hybrid sugarcane and showed worldwide regional distribution. The second group, including the maize or MZ group, which included 48 isolates, of which 47 isolates were infecting maize in different countries including China, Europe and Argentina, and 1 isolate was infecting sorghum in China. The third group, the noble sugarcane or NSCE group, was newly identified by Xu et al. (2008). The fourth group, the isolates from Thailand, which was named as the SCE/MZ group was given by Gemechu et al. (2006) and which included 19 isolates that were either infecting sugarcane or maize in Thailand. The fifth group was tentatively identified as the Brazil group by Xu et al. (2008). The two unique strains are SCMV-MDB from maize in the USA and SCBV-Abaca from Musa textiles in the Philippines. Xu et al. (2008) showed that the average CP gene nucleotide identities showed the diversity of SCMV within the group with the variation from the largest to smallest

is listed as SCE> Thailand> Brazil> MZ> NSCE group, with average identities of 95%, 94%, 92%, 88% and 87%, respectively (Xu et al. 2008).

14.3.5 *Sugarcane Yellow Leaf Virus* (SCYLV)

Sugarcane yellow leaf virus (SCYLV) belongs to the genus *Polerovirus* within the family *Luteoviridae*. Yellow leaf disease (YLD) affecting sugarcane genotypes exhibited yellowing of the midrib and lamina in different sugarcane growing states of India with a disease intensity of up to 100% in some of the susceptible varieties (Rao et al. 2000; Viswanathan 2002). In one of our study at Indian Institute of Sugarcane Research, Lucknow, we recorded the yellow leaf disease incidence from 5% to 55% in different sugarcane genotypes (65) and YLD affected genotypes exhibited various symptoms starting from smaller leaves and bunching at the crown region, yellowing of the midribs and followed by severe yellowing and necrosis from tips to downwards (Fig. 14.1d–e; unpublished data). SCYLV was identified as the causal virus of the YLD, which is transmitted through vegetative cuttings and one aphid species, viz. *Melanaphis sacchari* (Zehntner) (Viswanathan and Rao 2011; Singh et al. 2011). In India, association of SCYLV with YLD was reported by Viswanathan et al. (1999) for the first time. Besides diagnostics and molecular characterization of the SCYLV, the distribution led to study the genetic diversity. Moonan and Mirkov (2002) identified two groups of the virus among various viral isolates originating from North, South and Central America. One group contained only isolates originating from Colombia which was called as C-population and the second group called super population which was originated from Argentina, Brazil, Guatemala and USA. Subsequently, Borg et al. (2001) showed that the fingerprinting of the SCYLV sequence from various genotypes revealed diversity in its sequences both between, and within, the different geographic locations of the world. Based on the deduced amino acid sequence and phylogenetic analyses of SCYLV complete sequence, Abu Ahmad et al. (2006a) described the occurrence of three different genotypes viz., BRA, PER and REU from Brazil, Peru and Reunion, respectively from eight different virus isolates from different geographical locations. Subsequently, another virus isolate was reported from Cuba, which showed about 77–80% amino acid sequence identity in open reading frame one (ORF1) with these three genotypes reported earlier. Hence, this confirmed that the Cuban isolate representing as another genotype and designated as CUB genotype (Abu Ahmad et al. 2006a). Based on the close phylogenetic relationship, the two genotypes, viz. BRA and PER, were not differentiated and together they were called as BRA-PER (Abu Ahmad et al. 2006b). Moreover, in India Viswanathan et al. (2008b) and Singh et al. (2009) reported a greater variability between isolates originating from India and with the other isolates originating from different parts of the world. And a new SCYLV genotype IND was proposed, which is occurring only in Indian conditions. The isolate SCYLV-IND is closely related to the BRA, PER and REU genotypes. These studies suggested the presence of maximum variation in SCYLV populations and occurrence of maximum genetic diversity within the SCYLV

genotype (Borg et al. 2001; Moonan and Mirkov 2002; Abu Ahmad et al. 2006a, b). The complete genome of SCYLV genotypes showed 94.2–95.5% sequence similarities among SCYLV-A, F, IND, CHN-YL1, BRA-YL1, PER-YLb, REU-YLa, REU-YLb and REUYL2. Other virus isolates, with worldwide distribution, varied from 79.5% to 99.2% at aa sequence level. Most of the Indian isolates characterized were closely related to CUB genotype (Viswanathan et al. 2008b). Since, the Indian isolates shared closest sequence homology with the BRA and CUB genotypes with genetic variation within the Indian genotype, besides these two, IND was only found to occur in India (Viswanathan et al. 2008b; Singh et al. 2009). Besides the homogeneous population of Indian isolates, there was a great genetic variability existed among different isolates originating from different locations (Singh et al. 2009).

More recently, complete genome characterization of SCYLV with the evidence of RNA recombination from India was studied by Chinnaraja et al. (2013). Followed by the impact of SCYLV on the physiological efficiency from the tropical region of the country was rigorously worked out (Viswanathan et al. 2014). Moreover, the SCYLV symptom expression and variation in different sugarcane genotypes from tropical conditions were studied (Chinnaraja and Viswanathan 2015). The SCYLV-IND isolate showed 29.2–31.8, 28.1–34.4 and 30.7–33.4% sequence differences at aa levels with that of REU, HAW-PER and BRA based on the partial ORF0 sequence information, respectively. Likewise, based on the ORF1 partial sequence information it showed 21.4–23.7, 22.5–25.0 and 21.4–23.9% aa sequence variation with REU, HAW-PER and BRA, respectively. Based on the sequence identity of the complete genomes of IND isolates, it was found that the IND isolates are closely related to CHN1 genotype and shared 95.2–95.6% similarities, whereas it shared 86.3–86.6%, 86.1–86.7% and 84.9–86.2% similarities with REU, BRA and HAW-PER genotypes (Chinnaraja et al. 2013).

14.3.6 *Zucchini Yellow Mosaic Virus* (ZYMV)

Zucchini yellow mosaic virus (ZYMV), a member of the family *Potyviridae*, is a single-stranded positive-sense RNA virus with ~9.5 kb viral genome which encodes a single polyprotein precursor that cleaved into ten putative proteins (Gal-On 2007). ZYMV is considered to be an emerging important virus of cucurbits (Squash, Melon and Cucumbers) which achieved a worldwide distribution within two decades of its discovery and has the capacity to reduce agricultural yields up to 94% (Blua and Perring 1989). ZYMV infection often results in production of symptoms such as severe stunting, distinctive yellow mottling, blistering and lacination on the infected plants (Desbiez and Lecoq 1997). ZYMV infected plants often result in mottled and distorted fruits which are unmarketable. ZYMV is transmitted vertically by seed; however, it is less common than horizontal transmission through aphids, and it reported a seed-to-seedling transmission rate of 1.6% (Simmons et al. 2011).

A variety of studies have explored the extent and structure of genetic diversity in ZYMV, particularly within a bio geographical context. Based on nucleotide

sequence of 250 nt fragment, ZYMV isolates are majorly divided into two groups A and B, with the former divided into three clusters (Desbiez et al. 2002). Further analysis of the CP revealed three main groups of ZYMV isolates with different geographical distributions (Zhao et al. 2003). Group I includes the majority of the European isolates, some Japanese and Chinese isolates and a single California isolate. Group II are all from Asia (South Korea, Taiwan, Hangzhou and Japan), while group III includes several Chinese isolates. Members of all these groups differ in terms of the symptoms that they cause. Group I and II isolates cause the mosaic symptoms on leaves and fruit distortion whereas, group III viruses did not cause symptoms on fruit, but induced severe mosaic symptoms on the leaves (Zhao et al. 2003).

14.4 Mechanisms Responsible for Genetic Diversity

The two main mechanisms, viz. mutations, recombination or re-assortment, through which RNA viruses undergo rapid genetic changes and shuffle their genetic material with each other. Mutation creates nucleotide differences between the template and daughter strand during virus replication. In general, RNA viruses have high mutation rates due to error-prone RNA replication, large population sizes and short generation times, which result in sequence variants called a 'quasi-species' (Domingo et al. 1995; Domingo and Holland 1997). Several studies showed that plant RNA viruses are highly genetically stable and hence show a lower mutation rate than animal RNA viruses (Rodriguez-Cerezo et al. 1991; Malpica et al. 2002; Sanjuan et al. 2009; Tromas and Elina 2010). This stability might be due to strong bottlenecks during colonization of the host plant or during transmission by arthropod vector or weaker immune-mediated positive selection (Hall et al. 2001; Li and Roossinck 2004).

Genetic exchange by recombination or re-assortment is another source of genetic variation in virus populations. Recombination plays a very important role in shaping genetic diversity in some RNA viruses, particularly the retroviruses and a variety of positive sense RNA viruses (Worobey and Holmes 1999). During the recombination process, donor replaces a highly homologous region in the acceptor, which allows the recombination to retain the exact genomic organization of the parent RNA molecule. During the early studies of virus genome evolution, the importance of recombination was under-appreciated; however, it is now recognized as a widespread phenomenon among positive-strand RNA viruses (Holmes et al. 1999; Weng et al. 2007). Molecular characterization of full genomes of RNA viruses through sequencing has suggested that many natural strains of RNA viruses emerged from genetic recombination between related and unrelated viruses (Lai 1992). Thus, RNA recombination is being recognized increasingly as a significant and common phenomenon in the biology of RNA viruses (Mangrauthia et al. 2008). Re-assortment plays an important role in the evolution of RNA viruses with divided genomes. For example, CMV has been successful in adapting to various hosts and environments,

leading to an extremely large host range and a worldwide distribution (Chen et al. 2002; Bonnet et al. 2005).

14.5 Methods Used to Study Genetic Diversity

Consensus sequences generally represent average viral diversity within a population, so they are less informative of intra-host genetic diversity, masking the diversity of individual virions. However, most of the plant RNA viral genetic diversity studies had been conducted at the inter-host level and those examined at intra-host viral genetic diversity showed conflicting rates. In case of *Banana mild mosaic virus* (BMMV), it was observed higher levels of intra-host genetic diversity of more than 15% in a third of the sequence (Teycheney et al. 2005). Genetic diversity can be understood through serology-based assays, restriction fragment length polymorphism (RFLP) analysis, using host differentials and through next generation sequencing. RT-PCR-based RFLP was successfully employed to distinguish between SCMV and SrMV as well as between strains (Yang and Mirkov 1997). Nucleotide or amino acid sequence identity of the CP gene has been widely used for interpretation of genetic diversity for genus *Potyvirus*. Sequence identity percentages vary between 40% and 70% for different potyviruses and are above 90% for different strains of the same virus (Frenkel et al. 1989; Rybicki and Shukla 1992).

Use of differential hosts is the earliest method to study genetic variability by observing the plants for development of characteristic symptoms through mechanical inoculation of different strains. The virus strains differ in their host range, ability to cause infection and severity. However, the use of host differentials is time consuming and labour intensive and more importantly it does not reveal the viral diversity range. The characterization of symptoms produced on differential hosts is time consuming and reliable studies require the use of a set of standard differential hosts and previously described viral strains (Alegria et al. 2003).

Next generation sequencing represents an excellent tool for detection of allele frequencies present at low frequencies due to its very high level of coverage. This technique was successfully employed for understanding of intra-host genetic diversity in ZYMV infecting *Cucurbita pepo* (Simmons et al. 2012).

14.6 Seed-Transmitted Phytoplasma

As like plant viruses, phytoplasma diseases are also seed-transmitted. But the detailed information on the losses caused by seed-transmitted phytoplasma is not yet systematically studied so far. Earlier, certain reviews about the seed-transmitted phytoplasma did mention about seed-transmitted nature of the phytoplasma. For the first time, Bove et al. (1988) evidenced the possibility of symptoms of stubborn phytoplasma disease (*Spiroplasma citri*) in citrus seedlings caused due to seed-borne nature of phytoplasma. Moreover, it was not reported earlier that the *S. citri* is transmitted vertically through the seeds. Hence, the possibility of infection of

phytoplasma with seed coat cannot be ignored; the stubborn and corn stunt diseases were suspected to be a seed-transmitted which requires detailed study. The witches broom disease was reported to be seed-transmitted because disease was observed during the seedling stage of alfalfa (Khan et al. 2002). Subsequently, Cordova et al. (2003) observed the presence of phytoplasma in coconut embryos by PCR amplification. Hence, there was a possibility of coconut palms lethal yellowing disease transmitted through the seeds. Nevertheless, this needs further confirmation and detailed study about the role of seed transmission in the epidemiology of phytoplasma diseases. Later, Necas et al. (2008) reported the association of European stone fruit yellows phytoplasma (ESFY) as a seed-transmitted in apricot and observed very low seed viability of 21.6% in affected seeds. So far there are no reports of seed-transmitted phytoplasma in true seeds, and detailed and systematic study in this direction is required.

14.7 Conclusion

Knowledge of the plant viruses, genetic composition and viral strain identification at the genomic level are more crucial for the development of appropriate in vitro diagnostic tests and thereby efficient and stable control strategies. Still understanding of seed-borne and seed-transmitted viruses and phytoplasma is a grey area of research and needs detailed study on host-virus/phytoplasma interactions, distribution in host system and economic impact in various agricultural and horticultural crops, genome characterization, genetic diversity analyses and proper management approaches to reduce huge production losses. Moreover, the virus and phytoplasma are more diverse pathogens and causing severe damage to many economically important crops. Due to climate change and diverse cropping system, minor pathogens are becoming the major one; hence we need to join hands together to minimize the crop losses due to these serious pathogens. Collaborative efforts with national and international linkages are required in this direction. Plant viruses which are not reported as seed-borne or seed-transmitted need further investigation.

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Seed-Borne and Seed-Associated Nematodes: An Overview

15

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Abstract

Seed is the basic and critical input for crop production, and almost 90% of all the world's food crops are grown from seeds. Diverse groups of plant-parasitic nematodes are associated with variety of seed including seed tissues and/or propagating materials. Seed/propagating materials are the means for survival of plant-parasitic nematodes between growing seasons and also serve as effective means for introducing nematodes to new areas at local, regional and national levels. Some of the nematodes survive in true seeds in anhydrobiotic stage and survive for a long period in or on contaminated or infected seed. Consequently, they can be spread over vast distances in the commercial distribution of seed. Many of the plant-parasitic nematodes are known to survive and disseminate through edible roots, corms, rhizomes and tubers. The infested seed/propagating materials act as a source of inoculum for disease development that in turn cause both qualitative and quantitative losses to wide range of agricultural crops. In this chapter, economically important plant-parasitic nematodes associated with true seed, seed tissues and/or propagating materials are reviewed briefly.

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15.1 Introduction

Nematodes are multicellular, unsegmented, bilaterally symmetrical roundworms belonging to pseudocoelomate animals. There are over 4100 described species of plant-parasitic nematodes (Decraemer and Hunt 2006). Plant-parasitic nematodes are of great economic importance. Damage caused by plant-parasitic nematodes has been estimated at US$80 billion per year (Nicol et al. 2011). Nematodes alone or in combination with other soil microorganisms are known to attack almost every part of the plant including roots, stems, leaves, flower and seeds. Some plant-parasitic nematodes produce characteristic and recognizable symptoms of damage, but many of them only produce nonspecific symptoms and sometimes their presence is often only obvious when they have significant impacts on plant growth, development and yield.

The nematode life cycle is consisting of egg, four juvenile stages and the adult stage. The length of life cycle varies from a few days to nearly 1 year under optimal environmental conditions. Nematodes reproduce both sexually and by various asexual, parthenogenetic mechanisms. Plant-parasitic nematodes have a great variety of survival and dispersal strategies, which make them difficult to eradicate. The survival of plant-parasitic nematode between cropping seasons and its effective dispersal to uninfested plants are crucial aspects. Nematodes normally spread as soil-borne inoculum or in infected planting material. Some of the nematodes survive in true seeds in anhydrobiotic stage and survive for a long period in or on contaminated or infected seed. Consequently, they can be spread over vast distances in the commercial distribution of seed.

Seed is the basic unit in crop production technology. Seed plays an important role in associating many soil organisms including pathogenic plant-parasitic nematodes, which prove hazardous for seed or new plant created from it. Seed-borne/seed-associated nematodes play negative role in human welfare as well as agricultural production. The seed-borne/seed-associated nematodes affect the crop productivity in two ways. Firstly, seed and/or propagating materials containing viable nematodes introduce new pathogenic nematode species or races to new areas at local, regional or national levels. Secondly, introduced nematodes act as source of inoculum for disease development, which causes both qualitative and quantitative losses to a wide range of agricultural crops.

Few nematodes have been found to be true seed-borne mainly *Anguina tritici* in wheat, *Aphelenchoides besseyi* in rice, *A. arachidis* in groundnut, *Ditylenchus dipsaci* in broad bean, lucerne/alfalfa, onion, clovers, teasel and melon, *D. angustus* in rice and *D. africanus* in groundnut. Several nematodes are closely associated with seed tissue/propagating/planting materials. Many of them are known to be disseminated through edible roots, corms, rhizomes and tubers, namely, *Meloidogyne* spp. and *A. besseyi* on tuberose; *Radopholus similis* on banana; *D. destructor*, *Nacobbus* spp., *Globodera* spp. and *Meloidogyne* spp. on potato; *Pratylenchus coffeae*, *Meloidogyne* spp., *R. similis* and *Scutellonema bradys* on yams; *Meloidogyne* spp., *P. brachyurus*, *P. coffeae* and *Rotylenchulus reniformis* on sweet potato; *Hirschmanniella miticausa*, *P. coffeae* and *Meloidogyne* spp. on the roots of taro;

Meloidogyne spp. and *R. similis* in ginger; and *R. similis* in turmeric (Jatala and Bridge 1990; Lehman 1994). There are examples in which close association of nematode and seed tissues are known such as *A. ritzemabosi* in *Callistephus chinensis* (Brown 1956) and *Heterodera* in sugar beet. However, detailed information on true seed-borne transmission is lacking.

In this chapter, economically important plant-parasitic nematodes associated with true seed, seed tissues and/or propagating materials along with distribution, biology, symptoms and detection technique of true seed-borne nematodes are presented.

15.2 Nematodes Associated with True Seed

15.2.1 Seed Gall Nematode, *Anguina tritici*

Seed gall nematode, *Anguina tritici* is the earliest scientifically documented example of seed-borne nematode (Needham 1743). The nematode causes ear cockle disease in wheat and also a vector of a bacterium *Clavibacter tritici*, which is the causal agent of yellow ear rot or 'tundu' disease of wheat. The nematode also infects emmer (*Triticum monococcum*), ray (*Secale cereale*) and spelt (*T. spelta*). Barley (*Hordeum vulgare*) is a poor host. The nematode is known to cause severe crop losses in a crop, if poor agriculture practices including monoculture and poor-quality seeds (nematode contaminated seeds) are used. Presence of nematode galls along with healthy seed is the major source of inoculums.

15.2.1.1 Distribution

Present in all wheat-growing regions of the world including Europe, Asia, Africa, North and South America and Oceania.

15.2.1.2 Biology

After sowing of seed, the galls absorb moisture from soil and become soft. The nematodes become active, second stage juveniles (J2s) emerge from the seed gall in the soil and crawl onto newly germinated seedlings. The J2s reach growing tissue and are then carried up along with the growing seedlings. The juveniles feed ectoparasitically on growing tissue and subsequently enter the floral primordia. The J2s stimulate the formation of galls in place of seed development. Galls can develop from undifferentiated flower ovaries, stamen tissue and various other tissues. Progression through juvenile stages is completed inside the gall. Reproduction is amphimictic and newly formed adult female lays eggs, which hatch producing J2, which remain, encased in the galls as the survival stage and perpetuate plant infection in the following years. The dried galls are either harvested along with developed seeds or they fall into soil, and each gall may contain thousands of J2s. The J2s within drying galls can enter anhydrobiosis to survive during dry conditions. The total life period is between 105 and 113 days. The nematode completes one generation in a crop season (Gokte and Swarup 1987; Swarup et al. 1989; Mackesy and Sullivan 2016).

15.2.1.3 Symptoms

The first symptoms of nematode infection are basal swelling in the stem of 20–25-day-old infected seedlings. Nematode feeding causes crinkling, curling and twisting of leaves. Severe infection of young plants can result in stunted plants with distorted stems and leaves. The infected plants produce more number of tillers. Affected ears are short and broad with very short or no awns on the glumes. Infected ovaries are transformed into galls that are light brown to nearly black in colour which are shorter and thicker than healthy seeds (Swarup et al. 1989; Anwar et al. 2001; Bridge and Starr 2007; Mackesy and Sullivan 2016). The interaction of *A. tritici* with *Clavibacter tritici* in India results in an oozing from bacterial infected grains, known as Tundu disease or yellow ear rot.

15.2.1.4 Detection

To detect nematodes in dry seed lot, salt solution can be used. Seed are poured into a 20% salt solution, stirred vigorously, and the debris is skimmed from the surface and examined under the microscope for galls. To isolate the nematodes from the galls, which are dark brown-black in colour, smaller in size and irregular in shape, galls may be soaked overnight in water and teased under the microscope to remove the nematode larvae which emerge as a white cloud due to their large number. The Baermann funnel method may also be used to separate nematodes from gall in water. Any nematodes that emerge from seed will need to be confirmed using morphological/molecular methods (Mackesy and Sullivan 2016).

15.2.2 Rice White Tip Nematode, *Aphelenchoides besseyi*

Aphelenchoides besseyi, a rice white tip nematode is an important pest of rice that causes white tip disease. The disease is seed-borne and causes estimated losses about US$16 billion to rice crops (Lilley et al. 2011). As one of the ten most important plant-parasitic nematodes, *A. besseyi* has been found in most rice-growing areas of the world (Fortuner and Williams 1975; Duncan and Moens 2006; Jones et al. 2013).

15.2.2.1 Distribution

Rice-growing countries of Asia, Africa, North America, Central America, Caribbean, South America, Europe and Australia.

15.2.2.2 Biology

Aphelenchoides besseyi reproduces amphimictically although parthenogenesis can also take place. The nematodes can withstand desiccation and survive on stored seeds/grains in anhydrobiosis for several years. The nematode exhibits ectoparasitic behaviour with its infection rate matching the development of rice plants. After sowing, nematodes absorb moisture and become active and are attracted to meristematic areas and start feeding ectoparasitically on vegetative tissues. A rapid increase in nematode numbers takes place at late tillering and is associated with the

reproductive phase of the plant. Nematodes migrate to feed on reproductive structures eventually settling in the developing rice seed. The life cycle is generally completed in 8–12 days (Hoshino and Togashi 2000).

15.2.2.3 Symptoms

The characteristic symptom is whitening of the top 3–5 cm of the leaf tips which appears 15 days after emergence of seedling. The young leaves of infested tillers can be speckled with a white splash pattern or have distinct chlorotic areas. Leaf margin may be distorted and wrinkled but leaf sheaths are symptomless. The flag leaf enclosing the panicle crinkles and distorts, and the panicle is reduced in size, as are the grains. Infested panicles are shorter than normal panicles, with fewer spikelets and smaller portion of filled grain. (Dastur 1936; Todd and Atkins 1958). The panicles also often stay erect (Liu et al. 2008). The grain is small and distorted, and the kernel may be discoloured and cracked (Todd and Atkins 1958; Uebayashi et al. 1976).

15.2.2.4 Detection

The most commonly used methods for detection of *A. besseyi* is submerging seeds in shallow water after separating them from glumes (Uebayashi et al. 1971). Nematodes can also be extracted by Baermann funnel technique from seeds after they have been split and macerated (Nandakumar et al. 1975). Hoshino and Togashi (1999) developed a simple extraction method for determining the *A. besseyi* load on single rice seeds. The method is as follows: Individual rice seeds were split longitudinally and then transferred into single pipette tips. Tips containing a split seed were then singly placed upright in glass vials with water to extract the nematodes. This method has been found to be more efficient than the Baermann funnel technique and allowed nearly 100% recovery of living *A. besseyi* from single rice seeds within 4 h.

15.2.3 Groundnut Testa Nematode, *Aphelenchoides arachidis*

Aphelenchoides arachidis is a facultative endoparasite of the groundnut seed testa, pod shell, roots and hypocotyls and also feeds ectoparasitically on roots of groundnut (Bridge et al. 1977). The nematode was originally reported and described as damaging groundnut pods and seeds in Nigeria (Bos 1977) and is also reported on groundnut from South Africa (Lesufi et al. 2015).

15.2.3.1 Distribution

Nigeria and South Africa.

15.2.3.2 Biology

The nematode is both seed-borne and soil-borne. The nematodes are carried inside the seed and are released when seed absorbs moisture before germination. Pods are invaded 10 days after the fruiting pegs penetrate into the soil, but the number of nematodes in pods does not increase rapidly until after 30 days. The nematodes also

infect young roots and reproduce. All stages of nematode, including eggs, were found throughout the testae, but at the end of the growing season, heavily infested testae of mature seeds contains mainly juvenile stages with few adults (Bridge et al. 1977; Dickson and De Waele 2005).

15.2.3.3 Symptoms

The nematode lives endoparasitically in the testa of groundnuts and adversely affects the appearance and size of the seed. Translucent testae with dark brown-coloured vascular strands are observed in fresh seeds that are heavily infected, whereas dry seeds have wrinkled dark brown testae. Testae infested with *A. arachidis* are thicker and uneven than normal testae. Severely infested pods give smaller and shrivelled seeds. Seeds of infested pods may show reddish brown discolouration of seed-stalk after the germination (Bos 1977; Mc Donald et al. 1979; Lesufi et al. 2015).

15.2.3.4 Detection

The nematode can be extracted by soaking the shredded pod and seed tissues in shallow water in petri dishes for 24–72 h.

15.2.4 Stem Nematode, *Ditylenchus dipsaci*

Ditylenchus dipsaci is a migratory endoparasitic nematode which is one of the most devastating plant-parasitic nematodes and is widely distributed mainly in temperate areas. It attacks aerial parts, bulbs and tubers of plants, and its damage has been known since 1855 (Greco et al. 1991). More than 500 plant species from over 40 angiosperm families are known as hosts of this nematode. However, many of the various biological races of this nematode have a limited host range (Subbotin et al. 2005). This nematode is seed-borne in *Vicia faba* (broad bean), *Medicago sativa* (Lucerne/alfafa), *Allium cepa* (onion), *Trifolium* spp. (clovers), *Dipsacus* spp. (teasel) and *Cucumis melo* (melon) (Sousa et al. 2003: Sikora et al. 2005).

15.2.4.1 Distribution

Europe and Mediterranean region, North and South America, Northern and Southern Africa, Asia and Oceania.

15.2.4.2 Biology

Ditylenchus dipsaci is a migratory endoparasite which colonizes on parenchymal tissues where it feeds and reproduces. Each female lays 200–500 eggs. Duration of life cycle depends on temperature and differs among the isolates of different origins; however, it ranges from 14 to 23 days at 15 °C. Nematodes in all developmental stages are found throughout crop-growing season. The larvae hatch from eggs and quickly pass from second and third moult to the fourth-stage juvenile. Fourth juveniles tend to aggregate on or just below the surface of heavily infested tissue to form

clumps of 'eelworm wool' and can survive in dry conditions for several years (Hooper and Southey 1978; Griffith et al. 1997, 1999).

15.2.4.3 Symptoms

Symptom expression varies with host species, cultivars and environmental conditions. Infested stems are often swollen, stunted and distorted with malformed leaves. Necrosis or rotting of stem bases, bulbs, tubers and rhizomes are most common symptoms observed due to *D. dipsaci*. In broad bean, nematodes cause stem swelling and deformation of leaf tissues or lesions which turn reddish-brown and later black in colour. Leaf and petiole necrosis are also common under heavy infestations. Seeds infested with the nematode are darker, distorted and smaller in size and may have speckle-like spots on the surface (Sikora et al. 2005). In onion, nematode causes leaf deformation and leaf swellings or blister-like areas on the surface. The leaves grow in the disorderly fashion and often hang as if wilted. As the season progresses, they become chlorotic. Infested onions become swollen (bloat) and the bulbs may rot during storage (Sikora and Fernandez 2005). In lucerne/alfafa, nematode causes white discolouration of the stems and leaves termed as 'white flagging'. Stems of affected plants become thickened and shorter than healthy ones with the internodes reduced in length. Brown lesions can be observed externally and internally on infested stems. Heavily infested stems turn dark brown and may become dry and brittle. The buds of the infested plant are shorter and swollen and foliage had a shape similar to the spike of wheat (Lamprecht et al. 1987). In clovers, nematode causes thickening and twisting of the leaves, shortening and hypertrophy of the internodes and abnormal development of stolon and petiole epidermis. The stem tissue becomes spongy in texture and brittle (Grandison 1965; Cook and Yeates 1993; Griffith et al. 1997).

15.2.4.4 Detection

Nematodes may be present with, on or in the seed. Nematodes can be extracted by mist extraction system or by placing seeds on a Baermann funnel (preferably on a sieve covered with soft filter paper) and covered by a small amount of water. Larger seed samples can be submerged in water up to 4 days (Anonymous 2008). The legume and beet seeds can be separated from water on a 150 μm pore sieve and emerging nematodes can be concentrated on a 35 μm pore sieve before transferring to a counting dish (Green and Sime 1979).

15.2.5 Ufra Nematode, *Ditylenchus angustus*

The nematodes cause Ufra disease in rice and mainly occur in rainfed and irrigated rice ecosystems in seasonally deep flooded areas. Early season infection of nematode causes severe reduction in yield. Prasad and Varaprasad (2002) reported the nematode is a seed-borne as they recovered the live nematodes from the dried seeds (mainly located in the germ portion) after the storage period of 3 months. Previously, *D. angustus* had been found inside unfilled and filled spikelets of freshly harvested rice but not in dried seed (Butler 1919; Hashioka 1963; Sein 1977; Cuc and Giang 1982).

15.2.5.1 Distribution
Occurs in parts of India and Southeast Asia.

15.2.5.2 Biology
Ditylenchus angustus feeds ectoparasitically on the tissues of young or soft leaves and leaf sheaths and migrates upward to feed on newly forming tissues as the plant grows. All stages of *D. angustus* can invade rice seedlings. However, the J2 molts rapidly to J3. Thus, in nature, the J3, J4 and adult stages are likely the predominant invasive stages. Reproduction in *D. angustus* is amphimictic (sexual reproduction) with males as numerous as females within a population. The life cycle completes between 10 and 20 days at 30 °C, and at least three generations may occur within one growing season (Bridge et al. 2005a; Ibrahim and Perry 1994; Plowright and Gill 1994). At harvest, *D. angustus* is found in a desiccated state coiled up in crop residues, soil and seeds (Cuc 1982; Ibrahim and Perry 1993; Prasad and Varaprasad 2002).

15.2.5.3 Symptoms
Leaf chlorosis in infested young seedlings takes place within a week after infection. The chlorotic portion of leaf becomes brown to dark brown or mosaic discolouration of tender young leaves and sheaths especially near the leaf base. The twisting of leaf sheath is commonly found. The panicles may remain partially or completely enclosed within flag leaf or may emerge partially or fully from it. The fertility and grain filling are adversely affected and malformations of grain filling take place (Sehgal et al. 2001; Bridge et al. 2005a; Rahman 2003).

15.2.5.4 Detection
Separate the seed coat with endosperm and germ portion from each seed and incubate in petri plates containing 5 ml of water for 1 h and observe under a stereozoom microscope for the presence and enumeration of nematodes (Prasad and Varaprasad 2002).

15.2.6 Peanut Pod Nematode, *Ditylenchus africanus*

Ditylenchus africanus is one of the economically important plant-parasitic nematodes that limit groundnut production in South Africa (De Waele et al. 1997). It was first found in hulls and seed of groundnut in South Africa during 1987.

15.2.6.1 Distribution
The nematode is found in all peanut-growing areas of South Africa.

15.2.6.2 Biology
The temperature has a great influence on egg production, hatching and length of life cycle. The optimum temperature for egg hatch and egg viability (>90%) is 28 °C. Nematode completes the life cycle from adult to adult within 6–7 days (De

Waele and Wilken 1990). Therefore, many generations can be produced during a single growing season because of the short life cycle of *D. africanus* and favourable soil temperatures. Nematodes that survived in hulls and seed can re-infest and damage a subsequent groundnut crop, even from small initial population densities.

15.2.6.3 Symptoms

The first symptom is the appearance of dark brown tissues with a corky appearance at the pod base where peg joins the pod. Groundnut pods resemble black rot caused by the fungus, *Chalara elegans*. Infected seeds are usually shrunken, with dark brown to black micropyles and flaccid testae with darker vascular strands. The testae of infected seed can be easily removed by gentle rubbing and reveals a distinct yellow discolouration on its inner layer. Penetration of nematode at the base of the pod causes weakening of peg and pod connection that results in pod breaking off during lifting of the crop (De Waele et al. 1989). Infection of nematode results in decrease seed quality by increasing the number of unsound, blemished and soiled kernels (Mc Donald et al. 2005). In severe infections, the weakened pod may split open and second-generation seedlings may sprout around the mother plant.

15.2.6.4 Detection

D. africanus can be extracted from hulls and seeds of peanut by soaking the tissues in shallow water in petri dishes for 24 h at room temperature (Bolton et al. 1990)

15.3 Nematode Associated with Seed Tissues/Propagating Materials

Several plant-parasitic nematodes have been found to be associated with propagating materials. Many of them survive during the cropping season and serve as source of inoculum for disease development and spread to new areas.

The root-knot nematode, *Meloidogyne* spp., feeds and reproduces on corm tissue of tora and disseminate through seed corm and cormels. In ginger, cardamom, turmeric and tuberose, root-knot nematodes are disseminated through infested rhizomes or bulbs used for propagation. Rice white tip nematode, *Aphelenchoides besseyi*, is also known to survive and persist in tuberose bulbs (Khan and Ghosh 2011).

The false root-knot nematode, *Nacobbus aberrans*, is associated with potatoes and plays an important role in reducing the yield of potatoes in tropical and temperate region mainly in South America, North America and USSR. Planting of infected tubers is one of the major means of dissemination. The nematode invades the roots and initially causes small necrotic lesions followed by formation of small bead-like root galls. The nematode also penetrates the tubers to a depth of 1–2 mm below the skin surface but does not produce easily recognizable symptoms on tubers. Use of seed tubers free from *N. aberrans* contamination is one of the methods to prevent further spread and establishment (Manzanilla-Lopez et al. 2002; Bridge and Starr 2007).

Potato rot or tuber nematode, *Ditylenchus destructor*, occurs mainly in potato-producing countries particularly in temperature regions. The nematode invades the tubers and enters through lenticels on the skin surface. The infection of *D. destructor* results in formation of v-shaped areas of dry rot that extends well into the interior of the tubers. In advanced stage, tuber surface is marked with sunken, dark-coloured pits or skin cracks. Subsurface tissues develop a brown, matted, wool-like appearance. The nematodes survive and develop on infected tubers. Therefore, use of nematode free tubers is essential to prevent the dissemination (Bridge and Starr 2007; Anonymous 2008).

Yam nematode, *Scutellonema bradys*, is associated with yams and is an endoparasite of roots and tubers. The nematode invades young developing tubers through the tissues of the tuber-growing point, alongside emerging roots and shoots, and through cracks and damaged areas in the tuber skin. The nematode causes dry rot disease, which mainly occurs in the outer tissue of tubers extending up to 1–2 cm into the tuber. The initial stage of dry rot in the tuber tissue consists of cream and light yellow lesions immediately below the outer skin of the tubers. In the later stages of dry rot, infected tissue changes from yellow to light brown then turn dark brown to black in colour. External cracks also appear in the skin of the tubers and parts can flake off exposing patches of dark brown, dry rot tissues. The severe symptoms are seen in older and mature tubers. The nematode also feeds and reproduces in stored tubers after harvesting. The yams are propagated from small, whole tubers or pieces of tubers, which are principal means of dissemination of *S. bradys* (Bridge et al. 2005b; Coyne et al. 2005).

Lesion nematode, *Pratylenchus coffeae*, is a migratory endoparasite of yam roots and tubers. The nematode causes dry rot disease on tubers, and symptoms of tubers are very similar to those described for *S. bradys.* The nematode also reproduces and multiplies in stored yams and causes significant damage. The nematodes are mainly disseminated through seed tubers (Acosta and Ayala 1975; Bridge et al. 2005b). The nematode is also associated with a disease of tora and ginger and mainly disseminates through seed corms and rhizomes, respectively.

Radopholus similis, a migratory endoparasite, can be found in rhizome tissues of banana, ginger and turmeric and serves as source of inoculum for establishment and further spread.

Hirschmanniella miticausa is a migratory endoparasite of tora corms and roots, and causal agent of rot disease known as miti-miti disease. Nematode activity is mainly in corm tissues and causes rotting in corms. These nematodes are disseminated in diseased corm planting material; therefore, use of nematode-free planting material is most economical means of preventing nematode spread and damage (Bridge et al. 1983; Jatala and Bridge 1990).

Several plant-parasitic nematodes have been found to be associated with root stock of perennial crops and also ornamental plants which are primary means for distribution of nematodes to new areas. Lehman (1994) listed the nematodes that are frequently distributed through root stocks: *Hemicriconemoides mangiferae* on mango and lychee; *Heterodera fici* on fig; *Meloidogyne* spp. on citrus, cocoa, coffee, fig, guava, kiwi, papaya, passion fruit, pistachio and tea; *Pratylenchus coffeae* on

citrus and coffee; *P. loosi* on tea; *P. vulnus* on fig, peach and olive; *Radopholus* spp. on citrus and tea; *Rotylenchulus reniformis* on coffee, papaya and tea; *Tylenchulus semipenetrans* on citrus, olive and persimmon; and *Xiphinema index* on grapes.

15.4 Conclusion

Several plant-parasitic nematodes are associated with seed, seed tissues and/or propagating materials. Detailed nematological information on seed-associated nematodes is available for only few major agricultural crops. Therefore, greater attention is needed for the crops that are having greater local importance. The economic and sustainable means of preventing nematode damage is to prevent the spread of nematodes through seed and/or propagating materials to newer areas at local, regional, national and international levels. Efforts are to be made to prevent the spread of nematodes through exclusion, sanitation and quarantine which may contribute to future containment of nematode problems.

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Part VI

Major Seed-Borne Diseases in Different Agricultural Crops

Major Seed-Borne Diseases in Important Cereals: Symptomatology, Aetiology and Economic Importance

16

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Abstract

The seed-borne diseases are of utmost importance as it reduces seed quality, inflicts changes in the chemical composition of infected seeds, and renders grains unfit for consumption. Severe infection leads to considerable reduction in the viability of seeds. Despite advances in development of disease-resistant varieties and other management methods, many diseases of cereals like rice blast, brown spot of rice, Karnal bunt and head blight of wheat and Maydis leaf blight of maize continue to be the major cause of yield losses in crop production. Recent researches to understand the biology of major seed-borne diseases of cereals and their management for sustained productivity with integrated approaches are outlined in this chapter.

Keywords

Cereals · Symptoms · Seed-borne diseases · Biocontrol · Management

16.1 Introduction

Seeds are the best carriers of several pathogens which are responsible for most of the plant diseases leading to considerable loss of crop yield. The infected seed not only affects the quality of the grain but also spreads the seed-borne pathogens to different geographical regions. Many pathogens are carried by and move together with the seed having the potential to cause severe damage to crop production and crop seed for international trade once they are introduced. For the majority of seed-borne diseases (except loose smut of wheat and barley, bakanae disease of rice),

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spores are carried externally on the seed surface and pathogen survives in the seed coat, pericarp, glumes and endosperm. Yield losses caused by seed-borne pathogens in wheat are reported between 15% and 90% (Wiese 1987). Maize cultivation in the world is limited by diseases which cause grain loss of about 11% of the total production. The international exchange of cereal seed both by way of germplasm and commercial seed lots may pose considerable risk of introducing new strains or races of pathogen into areas previously known to be free. Therefore, it is important to know about seed-borne diseases of important cereals and their management.

16.2 Rice Diseases

Rice (*Oryza sativa* L.) is the most widely cultivated cereal crop in almost all parts of the world. Rice is known to be attacked by 56 fungal pathogens (Ou 1985), of which 41 are reported to be seed-borne (Richardson 1979, 1981). Wei (1957) also reported various fungal species infecting rice responsible for grain discoloration. Most fungi identified on rice are either (1) pathogenic fungi (causing typical characteristic disease symptoms) or (2) deleterious fungi (normally do not cause any disease but affect rice quality in storage, seed germination and cause seed rot). Bakanae, brown spot and blast are some of the widely distributed seed-borne fungal diseases of rice. A few diseases as leaf scald, sheath rot, false smut and kernel smut are of minor importance, but are increasingly gaining importance in the recent years. Three diseases account for most of the yield loss in rain fed lowlands are bacterial blight, blast and sheath blight. Major seed-borne diseases of rice along with the pathogens associated are listed in Table 16.1.

16.2.1 Blast Disease

Rice blast is a common problem occurring in rice-producing regions. The disease is reported from almost every rice-growing region of the world under uplands, lowlands and deep-water conditions, although severity is more under cooler climates. The disease continues to be a problem in many of the temperate and sub-tropical

Table 16.1 Major seed-borne diseases of rice and their pathogens (Mew and Gonzales 2002)

Diseases	Pathogens
Stackburn	*Alternaria padwickii*
Black kernel	*Curvularia* spp.
Sheath rot	*Sarocladium oryzae*
Leaf scald	*Gerlachia oryzae*
Bakanae	*Fusarium fujikuroi* (*moniliforme*)
Brown spot	*Bipolaris oryzae*
Blast	*Magnaporthe* (*Pyricularia*) *oryzae*
Glume blight	*Phoma* sp.
Kernel smut	*Tilletia barclayana*

rice growing areas because of unpredictable outbreaks and resultant economic loss. *Magnaporthe oryzae* is considered the most destructive of rice pathogens owing to its wide distribution, severity and economic loss caused by reduction in quantity and quality of the rice harvested. Losses due to blast have been quantified generally and its epidemiology studied in detail but it would appear that there has been less than systematic approach to researching or managing blast in the context of the rice ecosystem (Teng 1994). Blast epidemics are commonly known to be affected by climate, varietal susceptibility and crop management practices such as high nitrogen inputs and low soil moisture. Rice grown in the plateaus (Eastern India, Western Ghats of Karnataka, Maharashtra) and hills (sub-Himalayan region) during the *Kharif* season, especially (Basmati and other aromatic rice) rice grown in the foothills of Himalayas and the cool-season crop in Andhra Pradesh and Tamil Nadu, are endemic to blast in India (Variar et al. 2009). Blast pathosystem is known to have two major subsystems: the leaf and panicle blast pathosystems. Disease is initiated with primary inoculum present in plant debris or as alloinfection from infected rice or non-rice hosts. Disease intensity increases via autoinfection. Field epidemics of blast are polycyclic. The disease may appear as leaf blast, collar rot, node blast, panicle blast or rotten neck blast depending on the portion of the rice plant infected.

16.2.1.1 Symptoms

Magnaporthe oryzae infects and produces lesions on all aboveground parts of the rice plant including leaves, leaf collar, stem, nodes, panicles and grains. Shape, colour and size of the lesions vary depending on the site of infection, varietal resistance, age of the lesions and environmental conditions. Under favourable conditions, lesions enlarge and coalesce, eventually killing the leaves. Leaf blast lesions are elliptical or spindle shaped ranging in length from 1.0 to 1.5 cm and width of 0.3–0.5 cm and has brown margins with grey centre (Fig. 16.1). Some resemble a diamond shape and are wide in the centre and pointed towards either end. Dark lesions on the panicle neck node and on the flag leaf collar are commonly referred to as neck rot. Infected plants often display white, partly or completely unfilled panicles, a symptom confused with white ear heads caused by stem borers and can be found on the panicle branches, spikes and spikelets. Seeds are also infected. Collar rot occurs when lesions are located at the area of necrosis at the union of the

Fig. 16.1 Rice leaf blast disease symptoms

two tissues. Collar infections can kill the entire leaf and may extend a few millimetres into and around the sheath. The pathogen also infects the nodes that appear black-brown and breaks easily; this condition is called node blast.

16.2.1.2 Pathogen

Magnaporthe oryzae B.C. Couch (formerly *Magnaporthe grisea* Cavra) is a heterothallic, filamentous ascomycete, classified in the family *Magnaporthaceae*, producing three-septate spindle shaped, hyaline ascospores in unordered asci found within perithecia. The mycelium of *M. oryzae* is septate and the nuclei within the mycelium and spores of this fungus are haploid. The sexual stage is not found in nature. The asexual stage of the fungus is *Pyricularia oryzae*, the commonly found form produces three-celled conidia on the apex of conidiophores that extend beyond the surface of lesions and in the culture media. The conidia, produced abundantly under humid conditions, are dispersed by wind or rain splashes. Infection cycle of *M. oryzae* begins with the attachment of conidia on rice plant surfaces. Spore tip mucilage, found on the conidial apex, serves to attach the conidium to nearly any surface, even under water (Hamer et al. 1988). The fungus senses physical and chemical signals from the plant surface to trigger germination and pathogenic differentiation. Germ tubes produced from conidia differentiate into specialized infection structures called appressoria. Surface rigidity is a critical trigger for appressorium development. Appressorium formation is regulated by the interaction between cell-wall matrix proteins of the emerging germ tube and a hydrophobic surface which results in the accumulation of cyclic adenosine monophosphate (cAMP) in the fungus. Elevated levels of cAMP in turn trigger transductive pathways resulting in new gene expression leading to infection structure (appressorium) formation (Dean et al. 1994). The fungus penetrates the plant cuticle and cell wall using turgor pressure created in the appressoria. The penetration pegs then differentiate into primary infection hyphae which, in turn, develop invasive hyphae in plant cells. The rice blast fungus is a hemibiotroph with an initial biotrophic invasion phase followed by a distinct phase of necrotrophic killing of host cells. Kankanala et al. (2007) proposed that rice blast defines a new paradigm in hemibiotrophy in which each successive plant cell invasion is biotrophic, but invaded plant cells die as they fill with hyphae. During its further development, the fungus spreads through the intercellular spaces to reach the stomatal chamber. From there, melanin-containing conidiophores are released onto the leaf surface. Subsequent formation of spores completes the asexual cycle. The entire development cycle of rice blast takes about 4 days from inoculation to sporulation. Under favourable conditions for infection, sporulation is immediately followed by secondary infection and further spread of the disease.

16.2.1.3 Disease Cycle

The blast fungus can overseason from one season to the next on rice straw and stubble, but survival is not important for the pathogen in the tropics as conidia are present in the air throughout the year. Under favourable conditions, a single lesion can develop and produce conidia within a week and continue to sporulate for 2–3 weeks. Several such cycles are completed in the rice-growing season. When the

cycle is repeated many times, spore load is very high leading to an epidemic. The intensity of the epidemic is determined mainly by the abundance of conidia, influence of environmental factors on disease processes, susceptibility of the rice varieties under cultivation and crop management practices followed. The disease may progress through several phases starting with leaf blast and followed by collar, panicle and node blast. Leaf blast usually increases early in the season, and then declines later as leaves become less susceptible. The disease is favoured by long periods of high humidity, little or no wind at night and night temperatures between 18 and 24 °C. Leaf wetness from dew or other sources is required for infection. Spores are produced and released under high relative humidity (RH) conditions, with no spore production below 89% RH. Sporulation increases with increasing RH above 93%. The optimum temperature for spore germination, lesion formation and sporulation is 25–28 °C. Excessive nitrogen fertilization, aerobic soils and drought stress favour the disease. Ammonium nitrogen gets converted to nitrate nitrogen under aerobic/drought conditions which increase susceptibility of rice to blast.

16.2.1.4 Management

Destruction of infested crop residue to reduce inoculum, use of clean, preferably treated seeds, manipulation of planting time (early seeding during the wet season), judicious use of nitrogen in split doses and maintenance of soil moisture to prevent dry conditions are generally recommended cultural practices for blast management. Time of planting is important because the interaction between humidity, temperature and stage of maturity influences the susceptibility of the plant to blast. Technologies which may improve the efficiency of allocation of labour and draft power resources may assist resource-poor farmers to develop cultural practices to prevent rice blast (Zeigler 1994). The use of power tillers, zero till machines or fertilizer cum seed drills could improve the ability of farmers to control time of planting in relation to incidence of rice blast in direct seeded systems. Proper spacing should be used to prevent favourable microclimate for polycyclic disease development.

Disease management utilizing host plant resistance has been one of the major objectives in rice breeding. *Magnaporthe oryzae* is known for its genetic instability, allowing it to overcome the genetic resistance of host plants. In spite of this, plant resistance has still been the most-effective and economical control of the disease. In rice-blast pathosystem, race-specific resistance is governed by the gene-for-gene relationship (Kiyosawa 1971; Silue et al. 1992). To-date, about 85 major blast resistance genes and approximately 350 quantitative trait loci (QTLs) have been mapped on the rice genome (Ballini et al. 2008). Most of the resistance genes are concentrated in certain genomic regions, particularly on chromosomes 6, 11 and 12. Some of these genes could be identical or allelic, since very few allelism tests were performed. Several cultivars, such as 'IR36' and IR64, were found to be durably resistant showing that, in some cases, genetic resistance can provide effective long-lasting protection against blast. Sallaud et al. (2003) identified nine genes derived from IR 64 in a mapping population of a cross between IR 64 and Azucena. Some major genes, such as *Pi-1*(*t*) and *Pi-2*(*t*), have broad resistance spectra, and can prevent

disease development by most lineages of blast in several countries. Advances in molecular genetics and completion of the genome sequence of rice paved the way for cloning and characterization of a number of major genes for blast resistance. The cloned genes share structural similarity that bears signature motifs of nucleotide-binding sites (NBS), leucine-rich repeats (LRR) and kinases. Although only a handful of major R genes in rice have been cloned and functionally confirmed by the complementation experiments, resistance gene analogs (RGA) and markers linked to major genes have been widely used as marker-aided selection (MAS) tools (Liu et al. 2002; Ramalingam et al. 2003). Genes controlling partial resistance to blast, however, remain poorly documented. It has been proposed that specific partial resistance could be controlled by defeated complete resistance genes (Talukder et al. 2004). This mechanism was demonstrated for the complete resistance gene to bacterial blight *Xa4*. This gene shows residual resistance against virulent strains (Li et al. 1999). An alternative hypothesis could be that partial resistance is governed by defence genes. This hypothesis is supported by recent experiments showing the successful use of defence-gene-derived markers to improve selection for quantitative resistance to blast (Liu et al. 2004; Wu et al. 2004).

Diverse parents are used by the breeders in hybridization programmes, some of which have major genes and QTLs for resistance to blast. All the breeding lines originating in the country are exposed to blast in hot-spot locations under the coordinated network (Directorate of Rice Research, Hyderabad) and hence lines carrying effective genes get selected. The improved lines thus carry complete and partial resistance genes as a result of which severe epidemics have not been reported in recent years. Where the breeding cycles are few and efforts are made to retain the genetic constitution of the traditional ecotype, as in Basmati rice, blast has remained a recurrent constraint to achieve higher yields. Selections made in blast-free environments succumb to blast when grown in blast-endemic environments.

Among the bio-control agents, bacterial antagonists are considered ideal candidates because of their rapid growth, ease of handling and aggressive colonization. Bacterial antagonists, *Pseudomonas* and *Bacillus* in particular, are good candidates for biological control. *Bacilli* are gram-positive endospore-producing bacteria that are tolerant to heat and desiccation and hence effective for field application. The pseudomonads are gram-negative rods, have simple nutritional requirements, are excellent colonizers and widely prevalent in rice rhizosphere. Gnanamanickam and Mew (1992) collected more than 400 strains of bacteria antagonistic to *M. oryzae* in laboratory assays. Krishnamurthy and Gnanamanickam (1998) reported 59.6% suppression of rice blast in a field experiment using the strain *Pf*7-14 of *Pseudomonas fluorescens* which produce antifungal antibiotics that inhibit germination of conidia of the blast pathogen. Biocontrol agents can be applied either by direct inoculation (bacterial cell suspensions for seed treatment, soil application by a drip system or foliar sprays) or by the use of various solid-phase inoculants. Vidhyasekaran et al. (1997) developed a talc formulation using a fluorescent pseudomonad (*P. fluorescens Pf*1) which effectively controlled the disease and increased grain yield. Jaiganesh et al. (2007) reported that a talc-based inoculum of *Serratia marcescens* applied on the foliage at 2.5 Kg/ha controlled blast, apparently through chitinolytic

enzymes which cause degradation of the fungal cell walls, induction of plant defence reaction and certain antifungal low-molecular-weight molecules. Gnanamanickam (2009) documented in detail about biological control research in rice and described environment-friendly, affordable, microbe-based disease control measures for blast, sheath blight, bacterial blight and other fungal diseases of rice.

Fungicidal control of blast is useful when resistant varieties are not available or planted, the area is endemic to the disease and windows for cultural control are limited. Seed treatment to prevent infection of the seedlings after germination is a preferred method in endemic locations. One or two applications of fungicides to the foliage at tillering or at booting would further reduce the incidence of leaf and neck blast. Early generation chemicals like edifenphos, isoprothiolane and metominostrobin exhibited specific fungicidal effects on the pathogen. Widespread use of organo-phosphorus compounds often caused the development of resistance in the pathogen. Interest, therefore, shifted to non-fungicidal disease-controlling agents. Melanin biosynthesis inhibitors like tricyclazole, pyroquilon, carpropamid and thalide and plant defence activators like probenazole are non-fungicidal and hence less likely to lead to resistance problems. Tricyclazole and Carpropamid are root-systemic fungicides highly effective against rice blast. These chemicals inhibit scytalone dehydratase—an enzyme involved in fungal melanin biosynthesis—effectively preventing the pathogen from penetrating the host plant. Carpropamid not only inhibits melanin biosynthesis by the pathogen but also induces resistance in rice plants through induced lignification. Probenazole is absorbed by the roots, and then systemically transferred to the whole plant, almost completely controlling leaf blast for 40–70 days after application. It activates the phenylpropanoid pathway that plays an important role in the plant defence system. Despite extensive use of these chemicals over many years, no development of resistance in the target fungus has been observed. An unusual feature of these chemicals is their specificity for rice blast control. With few exceptions, they do not provide strong control of other rice diseases or of diseases on other crops (Froyd and Froeliger 1994).

16.2.2 Brown Spot Disease

Brown spot of rice is widespread and occurs in all rice-growing countries. The disease causes losses in stand due to seedling blight, in yield due to leaf and culm infection and in quality and yield by kernel infection. The most dramatic aspect of the disease recorded was that it was considered to be a major factor contributing to Bengal famine of 1942 (reported by the Famine Inquiry 1945, which was headed by Sir John Woodhead), the losses then amounting to 50–90% (Ghose et al. 1960; Padmanabhan 1973). The range of reported yield losses, which are very often reported in relative terms, is extremely variable: from 4% to 29% (Bedi and Gill 1960), about 12% (Aluko 1975), from 8% to 23% (Fomba and Singh 1990) and from 26% to 52% (Chakrabarti 2001). Yield losses caused by brown spot may be attributed to a number of damage mechanisms (Rabbinge and Vereyken 1980; Rabbinge and Rijdijk 1981), in addition to LAI reduction and presumably

self-shading of lesions on underlying canopy. These include an early senescence of the diseased plants (Klomp 1977), a reduction in number of tillers and a reduction in root and shoot length (Vidhyasekaran and Ramadoss 1973).

The disease is chronic, affecting millions of hectares worldwide every year (Savary et al. 2000a; Chakrabarti 2001; Zanão Júnior et al. 2009), in environments where (1) water supply is scarce, (2) imbalance in plant nutrition occurs or (3) soil chemical characteristics are conducive to disease. These conditions are associated with poor farmers' fields (Ou 1985). Brown spot is widely encountered across India (Reddy et al. 2010) and the neighbouring South and South-East Asian countries (Savary et al. 2000a), causing yield losses that, on average, are in the range of 10% of the attainable yield wherever it occurs (Savary et al. 2000b) in the lowlands of tropical and subtropical Asia. There is, further, clear indication that brown spot is becoming more frequent and more severe as drought is becoming more frequent (Savary et al. 2005).

16.2.2.1 Symptoms

Brown spot is a fungal disease that can infect both seedlings and mature plants. Infected seedlings have small, circular or oval, brown lesions, which may girdle the coleoptile and cause distortion of the primary and secondary leaves (Fig. 16.2). Infected seedlings become stunted or die. Black discoloration of the roots causes distorted seedlings. On mature plants, typical spots on the leaves are circular to oval with grey to light brown centre and reddish-brown margin. Black to dark brown spots may also appear on the glumes and when infection is severe, the entire panicle can turn brown. The fungus may also infect grains causing black spots and/or grain discoloration. Young or underdeveloped lesions on older leaves are small and circular, dark brown or purplish brown. A fully developed lesion on older leaves is oval, brown with grey or whitish centre with reddish brown margin. Lesions on older leaves of moderately susceptible cultivars are tiny and dark. When infection is severe, the lesions may coalesce, killing large areas of affected leaves. Infected glumes have lesions with velvety appearance, and grains may be discoloured with black spots.

Fig. 16.2 Brown spot disease of rice

16.2.2.2 Pathogen

Bipolaris oryzae (Breda de Haan) Shoemaker syn. *Helminthosporium oryzae* Breda de Haan, the anamorph of *Cochliobolus miyabeanus* (Ito and Kuribayashi 1927) Drechsler, is the most accepted name for the causal agent of brown leaf spot disease of rice. Early infection processes of this genus include conidial attachment to the host surface, appressorium formation and direct penetration of host cuticle using an infection hypha differentiated from the appressorium (Ou 1985). In addition, in the last decade, several workers showed the pre-penetration morphogenesis of *Cochliobolus* spp. and characterized some fungal genes involved in these developments (Braun and Howard 1994a; Clay et al. 1997; Horwitz et al. 1999; Ganem et al. 2004). Several *Bipolaris* species adhere non-specifically to surfaces (Pringle 1981; Evans et al. 1982; Braun and Howard 1994b). Two-layered extracellular matrix of germ-tubes of *Bipolaris maydis*, *B. zeicola* and *B. turcicum* has been visualized by histological staining (Evans et al. 1982; Braun and Howard 1994b). The inner layer, which probably contains proteins (Evans and Stempen 1986; Braun and Howard 1994b), has been shown to be responsible for adhesion of conidia to surfaces (Braun and Howard 1994b). Apoga and Jansson (2000) showed that the inner layer of *B. sorokiniana* germ tubes consists mainly of proteins, and the outer layer of carbohydrates. By using a mutant of *Cochliobolus heterostrophus*, defective in the outer germ-tube extracellular matrix layer, Zhu et al. (1998) showed that the two layers may exist independently and still adhere to the leaf surface; thus, the inner layer may be mainly responsible for the adhesion. Disruption of G-protein α and β subunits of *C. heterostrophus* resulted in the inhibition of appressorium formation (Degani et al. 2004; Ganem et al. 2004). In contrast with the results from the α subunit deletion, disruption of the β-subunit resulted in the reduction of fungal virulence on maize leaves (Degani et al. 2004; Ganem et al. 2004). Synchronous inhibition of appressorial development and disease implies that conidial germination and appressorium formation should be indispensable for the infection to take place and ubiquitous signal transduction cascades might participate in these morphogenetic events of *Cochliobolus* spp.

Mature *B. oryzae* conidia are multicelled and usually germinate bipolarly, i.e. by germ tubes from both polar cells and sometimes from one polar cell and intermediate segments or intercalary cells. Thus, a single spore may generate more than one infection point (Ou 1985). Optimal temperatures for conidial germination (25–30 °C) and hyphal growth (27–30 °C) have been reported between 25 and 30 °C (Nisikado 1923), corresponding to optimum temperatures for infection and lesion expansion (25–30 °C; Dasgupta and Chattopadhyay 1977). Increasing leaf wetness periods generally led to increased lesion densities (Imura 1940; Sherf et al. 1947; Percich et al. 1997). Padmanabhan and Ganguly (1954) demonstrated that the susceptibility of plant tissue increases with their age.

As in many similar necrotrophic foliar pathogens, the incubation period is very short sometimes even 24 h (Klomp 1977; Sarkar and Sen Gupta 1977). The infectious period is initiated rapidly (3–4 days; Van Ba and Sangchote 2006) but progressively, as sporulation reaches high levels about 6 days after infection (Sarkar and Sen Gupta 1977). Brown spot is generally not observed in years with normal rainfall

(Singh et al. 2005b), whereas seasons with limited rainfall but heavy dew are conducive to stronger epidemics (Sherf et al. 1947). Lower rainfall in 2002 however corresponded to higher severity (Pannu et al. 2005). Drought has been long recognized as a key factor favouring brown spot epidemics. Early studies showed that water shortage to plants was enhancing disease thus leading to more disease occurring in rain-fed than flooded plots (Kulkarni et al. 1979; Hegde et al. 1999). Overall, temperature does not appear to be a limiting factor for brown spot epidemics (Dasgupta and Chattopadhyay 1977). Temperature and humidity, in the form of leaf wetness, however interact on infection efficiency (Percich et al. 1997). This explains why decreasing daily minimum temperatures (9.3–7.5 °C) lead to more severe epidemics (Minnatullah and Sattar 2002).

The literature dealing with soil characteristics, plant nutrition and physiology and brown spot disease is quite large and diverse. This literature also addresses the interactions between plant (hydro) mineral nutrition with a range of other factors influencing the disease, making a summary difficult to develop. Only some main features are outlined here. Brown spot has long been associated with soil fertility (Ou 1985; Damicone et al. 2001), for which the disease sometimes is considered a marker. Ou (1985) concluded that the main factor governing brown spot is the physiological condition of the rice plant, which is strongly influenced by the soil characteristics, especially with respect to N, K, Mn, Mg, Si, Fe and Ca. Similar conclusions were drawn by Damicone et al. (2001). Emphasis has often been on deficiencies, e.g. of N (Misawa 1955; Phelps and Shand 1995), of K (Padmanabhan et al. 1962), of P (Singh et al. 2005b), of P and K (Chattopadhyay and Chakrabarty 1965), of N or K (Spradley et al. 2003; Carvalho et al. 2010) or of Si, Mn and K. However, one important element is the interactions amongst these elements (Ou 1985; Chakrabarti 2001), which may partly explain the occurrence of optimum response patterns (as in the case of N; Chattopadhyay and Dickson 1960). For instance, interaction between N and P is documented by Phelps and Shand (1995), with susceptibility showing an optimum pattern as a function of P supply if the N supply is near-optimal. Further, the fact that some elements can be leached from the soil more easily than others (Chakrabarti 2001), brings about a link between soil nutrient effects with both soil texture and rainfall patterns. One additional element is that the effects of nutrients depend on the redox potential of the soil, which has been described in numerous reports (Ou 1985). One element, Si, appear to stand apart from the others, as Si-based amendments appear to address one of the main causes for susceptibility, as well as one avenue for the management of brown spot (Datnoff et al. 1991), as well as of other rice diseases. Recent results in the silicon uptake (Dallagnol et al. 2010) process may also open avenues towards breeding for more efficient Si uptake, and general resistance. Mineral nutrition is related to water uptake (Allaway 1968; Gardner et al. 1985). A shortage of water may result in reduced availability of certain micronutrients (Weil and Holah 1989). Water supply also influences the pattern of brown spot response in rice to nutrients, as in the case of P, which follows an optimum curve when water is scarce (Singh et al. 2005b). A very complex set of interactions between water supply, nutrients, soil factors and brown spot therefore are at play. Three elements appear to be critical for a better understanding of brown

spot epidemics and their management: the role of nutrients in disease predisposition, the effect of drought (in interaction with the former element), and the interplay of the first two elements with soil characteristics on the hydro-mineral plant nutrition.

16.2.2.3 Disease Cycle

The available information regarding the nature and importance of different sources of inoculum is patchy and mostly non-quantitative. According to Sharma and Maheshwari (1982) the primary infection is from seed and secondary infection from air-borne inoculum generated by infested debris and lesions. On the other hand, Biswas et al. (2008) reported that seed transmits the disease, and also that soil, and some weed host may serve as reservoirs. The fungus can survive on infected rice straw and stubble (Ou 1985; Sato et al. 2008) and can survive on husks and in the kernels for more than 3 years (Gangopadhyay and Chakrabarti 1987). Aside from the rice plant, the disease also infects barley, oats, *Cynodon dactylon* (L.) Pers., *Digitaria sanguinalis* (L.) Scop., *Eleusine coracana* (L.) Gaertn., *Leersia hexandra* Sw., *Panicum colonum* (L.) Link, *Setaria italica* (L.) P. Beauv., *Triticum aestivum* L. em. Thell. (wheat), *Zea mays* L. (maize) and *Zizania aquatica* (wild rice).

Mew and Gonzales (2002) reported that *Bipolaris oryzae* was often observed on the entire seed surface (about 32%) or on sterile lemmas (29%). Nyvall et al. (1995) reported that *Bipolaris* spp. were isolated primarily from the awns and frequently from the palea or lemma. The incidence of seed-borne *Bipolaris* spp. was related to disease severity in the field. According to Neergaard (1977) establishment and development of an infection within a seedling was linked in the process of seed transmission. Seed-borne nature and transmission of *Bipolaris oryzae* was also reported by Van Ba and Sangchote (2006). They investigated the relationship between disease severity on flag leaf and kernel infection, at three growth stages of flowering, milky and dough stages. Transmission of *Bipolaris oryzae* and location in the seed were studied, and each part of infected kernel including the embryo, endosperm, palea, lemma, rachilla and sterile lemma was found infected by *B. oryzae.*

16.2.2.4 Management

Strategies for brown spot control are limited because commercial cultivars with a high level of resistance are not available to the growers (Ou 1985). Consequently, fungicide application and proper plant nutrition have been the major management strategies available to growers (Ou 1985). It is known that rice plants grown on soils deficient in potassium, manganese, magnesium, silicon, iron or calcium are more susceptible to brown spot (Kaur and Padmanabhan 1974; Lee 1992). Silicon (Si) application to the soil offers a viable method to control brown spot, especially where soils are low or limiting in plant-available Si (Datnoff et al. 1991, 1997). Some economically important diseases in rice, such as blast, sheath blight, stem rot, leaf scald, leaf blight and grain discoloration, had their intensities reduced by Si application (Datnoff et al. 1997). Datnoff et al. (2007) reported a significant reduction in the severity of brown spot in rice plants growing in Si-deficient Histosol amended

with calcium silicate slag. Other investigators have also reported a reduction in brown spot severity by Si application (Nanda and Gangopadhyay 1984; Takahashi and Hino 1978). Physiology and pathogenicity of *Cochliobolus miyabeanus* Drechsler ex dastur (*Helminthospora oyzae* Breda de Hann) on rice have been studied; however, the mechanism of disease resistance is not yet fully understood. Ganguly and Padmanabhan (1962) recognized two types of resistance: resistance to prepenetration, and resistance to ramification and prepenetration. The former was attributed to mechanical characteristics of the epidermis and the latter to physiological defence in the protoplasm. Mishra and Prasad (1964) indicated that structural defence might be related to resistance. However, Chattopadhyay and Chakrabarti (1957) found no correlation between toughness of epidermal cells and resistance to disease. Oku and Nakanishi (1962) and Oku (1965) suggested that phenolic compounds and a phytoalexin like substance might be involved in resistance. Trivedi and Sinha (1978) found a fungitoxic substance in infected tissue. Hau and Rush (1982) reported that there was no significant difference in conidial germination on resistant and susceptible cultivars, germ-tube was longer on resistant plant where as it was short on susceptible cultivar and appressoria were produced earlier.

The search for sources of resistance to brown spot is a long-standing effort (Nagai and Hara 1930; Chakrabarti 2001), which still continues today. For instance, working on *Oryza sativa* species Satija et al. (2005) identified 15 entries out of 124 which were classified as resistant (less than 5% severity). Conversely, Hossain et al. (2004) identified one resistant variety out of 29 entries. It however is felt that the sources of resistance amongst *Oryza sativa* entries are few, and further research (Goel et al. 2006) has been exploring other pools, especially *O. nivara*.

Early studies showed that resistance or susceptibility could be associated with a limited number of genes. For instance, Balal et al. (1979) found that two dominant genes were associated with resistance, while one gene was associated with susceptibility. Adair (1941) suggested that resistance was recessive, involving several genes. Anatomical characters like thicker epidermal cuticles of the leaves and distribution of greater number of silicated bulliform cells were observed to be associated with traditional tall indica cultivars like Patnai-23, Bhasamanik and Tilakkacharry, which were less susceptible to brown spot disease. Goel et al. (2006) analysed the inheritance of brown spot resistance from crosses involving *O. nivara* germplasm and hypothesized that additive, dominant as well as epistatic gene interactions were involved. Three quantitative trait loci (QTLs) were detected in cvr. Tadukan (*qBS2, qBS9, qBS11*), located on chromosomes 2, 9 and 11, respectively (Sato et al. 2008), the latter (*qBS11*) being considered as having a major effect on brown spot resistance. However, Katara et al. (2010) identified 10 QTLs, some of which may be common to the results of Sato et al. (2008). De Vleesschauwer et al. (2010) have shown that exogenous abscisic acid treatment protects rice from infection by the brown spot pathogen *C. miyabeanus* through induction of a multilayered defence response. Besides modulating *C. miyabeanus*-induced ROS formation and requiring intact Gα signalling, they further demonstrated ABA to antagonize pathogen-activated ethylene signalling via the MAPK gene OsMPK5. Moreover, reports of

De Vleesschauwer et al. (2010) and results of several others published previously (Singh et al. 2004; Iwai et al. 2006; Bailey et al. 2009) highlighted the fine control of rice defences to *C. miyabeanus* and the blast pathogen *M. oryzae* through the differential engagement and balance of the abscisic acid and ethylene response systems.

Biological control is an innovative, cost-effective and ecofriendly approach. *Trichoderma viride* is known for its mycoparasitic and antagonistic mechanism for the control of several fungal diseases. Gomatinayagam et al. (2010) reported the efficacy of *T. viride* in restricting the growth of *Bipolaris oryzae* in dual culture. Abdel-Fattah et al. (2007) reported that under field conditions, spraying of spore suspension of *T. harzianum* at 10^8 spore ml^{-1} significantly reduced the disease severity and disease incidence on the plant leaves and also significantly increased the grain yield, total grain carbohydrate and protein and led to a significant increase in the total photosynthetic pigments (chlorophyll *a* and *b* and carotenoids) in rice leaves.

Brown spot control has been achieved in India, earlier with copper fungicides and later with organo phosphorus compounds like Dithane M-45. Fungicides, such as iprodione, propiconazole, azoxystrobin, trifloxystrobin and carbendazim (Moletti et al. 1996; Cortesi and Giuditta 2003; Mandal and Jha 2008), are also effective means to control the disease. The disease is consistently associated with unfavourable soil conditions and deficiency of plant nutrients, and it is generally believed that soil amelioration and correction of nutrient imbalances would lead to good control of the disease.

16.2.3 Bakanae Disease

Bakanae, also known as foot rot, is an emerging seed-borne disease of rice caused by the fungus *Fusarium fujikuroi* (Nirenberg) [telomorph: *Gibberella fujikuroi* (sawada) Ito]. It is one of the newly emerged increasing problems of rice, particularly in Basmati rice in India during recent years (Pannu et al. 2012). It is one of the major fungal diseases of rice including blast, sheath blight, brown spot and sheath rot in India (Sharma and Thind 2007). Crop losses caused by the disease may reach up to 20% in outbreak cases. In Japan, a 20–50% loss was observed. Bakanae is, therefore, emerging as a serious disease of rice in India, Japan, Taiwan and Thailand (Webster and Gunnell 1992; Kini et al. 2002; Saremi 2005; Bashyal et al. 2014). It is a major concern in the affected rice-growing areas of India and also becoming more alarmed threat for sustainable rice production in other parts of the rice-growing world. It is difficult to develop bakanae-resistant rice varieties due to the high genetic variation of the causal pathogens (Serafica and Cruz 2009). Although both blast and bakanae are becoming a threat for sustainable rice production due to high genetic variation of the causal pathogens, bakanae is more alarming compared to blast as the disease is not influenced solely by environment and mainly infection increases through ‘passive transmission’ from the infected seeds (Fig. 16.4).

16.2.3.1 Symptoms

Fusarium fujikuroi causes different types of symptoms starting from pre-emergence seedling death to grain infection at maturity; elongation than normal growth (Fig. 16.3), stunted growth (Ou 1985). The disease occurs both in seedbeds and in the field. In the seedbed, the grains fail to germinate or the seedlings fail to emerge above the soil. This type of pre-emergence damage occurs only to a small extent. Growth of the pathogen could be visible at the junction of the palea and lemma of the damaged grains (Thomas 1934). The mortality of the seedlings after emergence is often high in the nursery. These symptoms are noticeable from the sixth day after sowing in wet nurseries and may continue up to the sixth week. Such seedlings wilt and die within 3–6 days after the symptoms appear. All the affected seedlings may not exhibit overgrowth. Some plants which are apparently healthy may show the symptoms after transplant. Older diseased plants invariably produce fewer tillers than healthy ones. This difference is pronounced in the seventh and eighth weeks, when the tillering is at its maximum. The difference reduces in the later stages. As the flowering period approaches, the affected plants have lean and lanky tillers. On splitting open the culms of infected plants, brown discoloration of the nodal tissues is visible. In the initial stages this is confined to the lower nodes. In advanced phases the upper nodes also exhibit such symptoms. Inside the hollow internode, mycelial masses bearing spores are present. Later the fungal growth appears outside, in the basal nodal zones, producing a white or pink bloom on the surface.

Another symptom noticed between the seedling and tillering stages is the development of adventitious roots from a number of the lower nodes of the culm, above the ground-level. These may be partially or completely enclosed within the leaf-sheath. In healthy plants such roots are seen only from the first node above the ground. Further in some instances abnormal growth of whorls of root takes place at the collar region, imparting a woolly fasciculate appearance to the root system.

Fig. 16.3 Bakanae disease elongation symptoms

16.2.3.2 Pathogen

Three mating populations of section Liseola (A, C and D) of the *G. fujikuroi* complex have been associated with bakanae disease of rice. Mating population C (MP-C) (anamorph, *Fusarium fujikuroi*) (Nirenberg 1976), was first identified in 1977 among strains from rice from Taiwan. It has been found responsible for bakanae disease in Italy (Amatulli et al. 2010). Mating population A (MP-A) (anamorph, *Fusarium verticillioides* (synonym, *F. Moniliforme*) and mating population D (MP-D) (anamorph, *Fusarium proliferatum*) have been isolated from rice from Asia, and MP-D has been isolated from rice from Africa, Australia and the United States (Desjardins et al. 1997; Amoah et al. 1996). Thus, more than one species of *Fusarium* may be able to infect rice and cause symptoms of bakanae disease. In India three *Fusarium* spp. such as *F. fujikuroi, F. proliferatum* and *F. verticillioides* were found associated with bakanae disease of rice. Bashyal et al. (2014) assessed the distribution profile, characterized and identified the different isolates of *Fusarium* spp. associated, with bakanae in Basmati varieties. The incidence of *Gibberella fujikuroi* species complex (GFSC) varied from 1% to 24% in India (Bashyal and Aggarwal 2013).

16.2.3.3 Disease Cycle

The pathogen is seed- as well as soil-borne. The seed-borne inoculum is the main source of the disease as the soil-borne inoculum is quickly reduced under field conditions (Kanjanasoon 1965; Sun 1975; Ou 1985). In diseased rice fields, 100% of seed showed the pathogen and 30% of them produced bakanae symptoms when planted. Hino and Furuta (1968) reported the embryo infection of 8.85%, 8.1% in and 0.03% in July, August and September respectively. Kernels developed a reddish discolouration in severe condition, as there is presence of the conidia of fungus. Nishikado and Kimura (1941) observed the pathogen inside the xylem element. Seto (1937) resolved that flowering time is mainly favourable for the development of seed infection. Kanjanasoon (1965) observed 93% disease instantly in artificially inoculated soil after inoculation, in Thailand (Fig. 16.4).

16.2.3.4 Management

This disease is an increasing threat since no proper control measures are adopted. *F. fujikuroi* is both a seed-borne and soil-borne pathogen of rice which infects the seedlings at the time of germination or at an early growth stage through the roots or crown (Ou 1985). It has been observed that untreated seeds were heavily infested with an overall 94.5% with bakanae disease, whereas disease incidence in treated nursery was only 5.5%. Sun and Snyder (1981) reported that healthy rice seedlings may be infected sporadically after transplanting in the field. As the disease is primarily reported to be seed-transmitted (Webster and Gunnell 1992), seed treatment with Carbendazim (0.1%) represents the first way to control the spread of the disease. Seedling treatment and seedbed treatment with Carbendazim prevents soil-borne infection after transplanting.

Biocontrol agents viz., *Trichoderma harzianum, Talaromyces flavus, Chaetomium globossum, Pseudomonas fluorescens* were observed effective against bakanae

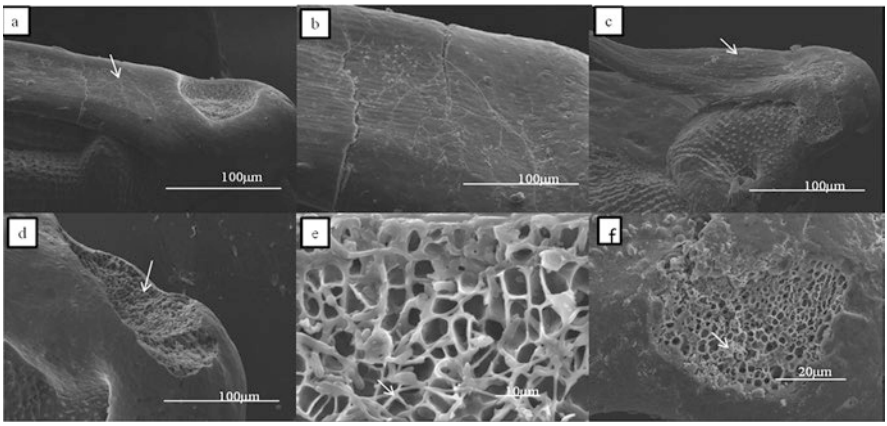

Fig. 16.4 Scanning micrographs showing colonization of glume and embryo by *Fusarium fujikuroi*. **a**, **b** and **c**, colonization of glume of rice seed PB 1509—**a**, **b** of 24 hpi and **c** of 72 hpi—and **d**, **e** and **f**, colonization of embryo after 24 and 72 h of inoculation, respectively

disease of rice in different studies. *T. flavus* produced high concentration of protease enzymes which appear to be more effective (Haggag et al. 2006).

Although the disease attacks both scented and non-scented groups of paddy, it is more common in high yielding dwarf and aromatic tall varieties (export quality). Rice varieties BR 1067-84-1-3-2-1, BR 1257-31-1-1, BR 4367-8-11-4-9, IR 58109-109-1-1-3, IR-6, DR-82, ADT-40, ADT-44, ADT-41, ASD-16, Amulya, Sabita, Ereimaphou, Prasanna, GR-4, IR-64, Akutphou, MTU-1010, Pusa Basmati 1121 were observed highly susceptible to the bakanae. However, disease was observed in other Basmati varieties also. In the year 2012, rice varieties Pusa 1401, Pusa 2511, CSR 30, Dehradoon Basmati and Pakistani Basmati rice varieties BR 1067-84-1-3-2-1, BR 1257-31-1-1, BR 4367-8-11-4-9, IR 58109-109-1-1-3, IR-6, DR-82, ADT-40, ADT-44, ADT-41, ASD-16, Amulya, Sabita, Ereimaphou, Prasanna, GR-4, IR-64, Akutphou, MTU-1010 were identified with different degree of resistance in India and Pakistan against bakanae disease.

16.3 Wheat Diseases

Wheat (*Triticum aestivum* L.) is one of the major cereal crops cultivated worldwide and is not only a staple food but also has increasing global demand for wheat based products. The world acreage under wheat crop is 222.28 mha with production of 727.2 mt with average yield of 3254 Kg/h. After China, India is the second leading producer of wheat in the world. In 2014–15, the country produced 94.70 mt from 31.2 mha with productivity of 3075 Kg/ha. Despite of this achievement in wheat production, to fulfill the food grain obligation of India, country necessities to produce 109 mt of wheat by 2020 (Nagarajan 2005). Wheat cultivation, which was earlier confined to rainfed situation, has extended to irrigated systems. Breeding

Table 16.2 Major seed-borne diseases of wheat and their pathogens

Disease	Pathogen
Karnal bunt	*Tilletia indica*
Head (ear) blight or scab	*Fusarium* spp.
Loose smut	*Ustilago nuda* var. *tritici*
Spot blotch	*Bipolaris sorokiniana*
Alternaria leaf blight	*Alternaria triticina*
Tundu or ear cockle or yellow ear rot disease	*Clavibacter tritici* and *Anguina tritici*

efforts and irrigation has increased cropping intensity, thereby supporting the spread of the diseases like rusts, loose smut, Karnal bunt and spot blotch. The major impact of seed-borne diseases in wheat is not only the yield reduction but also deterioration in market quality of grains. Infected wheat seeds are the carrier of pathogens for long distance dissemination. Detection and management of seed-borne diseases through quality-control programmes that monitor seeds from harvest to purchase, marketing and sowing in the field are essential to ensure high-quality, pathogen-free and genetically pure seed. Major seed-borne diseases of wheat are Karnal bunt, *Fusarium* head blight, loose smut and spot blotch (Table 16.2).

16.3.1 Karnal Bunt

The Karnal bunt is native to South Asia and was detected in experimental wheats grown at the Regional Station of IARI at Karnal, Haryana in 1930 (Mitra 1931). The disease is known by various names such as new bunt (Mitra 1931), Karnal bunt (Mundkur 1943) and partial bunt (Bedi et al. 1949). The disease which was confined to isolated pockets in the Indian sub-continent till early 1970's, has now occupied large areas in North-western region of the country. It occurs in an endemic form in the states of Punjab, Haryana, Jammu region of J&K, parts of Himachal Pradesh, Uttar Pradesh, Delhi, northern Rajasthan, Bihar and West Bengal. So far the disease has not been recorded in south of Madhya Pradesh, Maharashtra, Orissa, Assam, Karnataka, Andhra Pradesh, Tamil Nadu and Kerala (Joshi et al. 1983). Besides India and Pakistan, Karnal bunt is reported from Syria (Williams 1983), Afghanistan (Loke and Watson 1975), Nepal (Singh et al. 1989), Iran (Torabi et al. 1996), Mexico (Duran 1972), USA (Ykema et al. 1996) and South Africa (Crous et al. 2001). It has also been intercepted in wheat samples imported into India from Lebanon, Sweden and Turkey (Nath et al. 1981).

The occurrence of Karnal bunt is sporadic in nature but becomes serious in epidemic years and has caused substantial losses to wheat crop. McRae (1933) estimated loss up to 20% in a number of wheat cultivars. Loss estimates recorded in India reveal that the disease reduced annual yield of wheat by 0.2% in Punjab and Jammu (Munjal 1976). These estimates of losses have been confirmed by data collected through Wheat Disease Surveys conducted by IARI, New Delhi since 1975. Data have shown that even during worst years of epidemic, the total damage to the

crop was not more than 0.2–0.5% of the total production (Joshi et al. 1983). The yield loss is related to the size of bunt sori produced by different varieties (Bansal et al. 1984). The quality of wheat is adversely affected due to bunt infection. Flour milled from seed with 10% infection has dark colour. Since the Karnal bunt produces a volatile substance-trimethylamine, the palatability of 'chapaties' is reduced due to fishy odour and perceptible discolouration. The chapaties prepared from milled flour with 3% infected seed lot are unpalatable (Mehdi et al. 1973).

The importance of Karnal bunt vis-à-vis export of wheat is well understood. There is quarantine restriction by several countries on movement of wheat grains from affected areas. Agriculture Department of Canada has maintained zero tolerance for the pathogen (Martin 1986). European Union has also devised quarantine procedures to prevent introduction of the pathogen (Anon 1991). With in North America, zero tolerance level to Karnal bunt is followed to prevent movement of contaminated seed (Babadoost 2000). The movement of wheat grain from disease prone areas to other parts of Mexico is prohibited unless fumigated with methyl bromide (Fuentes-Davila 1996). On account of such implications of disease, export in India suffers considerably (Singh and Srivastava 1997).

16.3.1.1 Symptoms

Karnal bunt pathogen infects wheat at the flowering stage prior to seed formation, hence the symptoms are visible only when the grains have fully developed in ear heads. In a stool all the ear heads are not affected and also all the grains in a spike are not infected. A careful examination of individual ear reveals bunt infection in the field. In the standing wheat crop, the infected spike can be detected by the shiny silvery black spikelets, with glumes spreading apart and swollen ovaries. The spikes of infected plants generally are reduced in length and in number of spikelets (Mitra 1937).

The pathogen converts the infected ovary into a sorus where a mass of dark brown coloured teliospores are produced. Infection of grains varies from small sori to completely bunted seeds (Fig. 16.5). Small sori are generally developed in longitudinal furrow, leaving the dorsal side of the seed and endosperm unaffected. If the host is highly susceptible, the whole endosperm material may be converted into a large sorus, and seed looks hollow leaving only the pericarp and the aleurone layer.

Fig. 16.5 Karnal bunt disease

The grains are usually partially bunted and completely infected ones are rare (Mitra 1935; Chona et al. 1961). Therefore, the disease is also referred as 'Partial bunt' (Bedi et al. 1949). The infected ears emit a fishy odour due to trimethylamine, a volatile compound produced due to pathogenesis. In such cases the shrivelled embryo is dead.

16.3.1.2 Pathogen

The pathogen of Karnal bunt was first described by Mitra (1931) as *Tilletia indica* Mitra. Later Mundkur (1940) argued that the fungus should have been assigned to genus *Neovossia,* as it produces numerous no-fusing sporidia, hence he renamed the fungus as *N. indica* (Mitra) Mundkur. Fischer (1953), limiting the species of *Neovossia,* again referred it to *T. indica* given by Mitra. Duran (1972) also proposed that *T. indica* is correct taxonomic name of the fungus.

Teliospores of *T. indica* are dark brown to black, globose to sub-globose in shape having hyaline sheath of 2–4 μm thickness and measure 22–49 μm in size, average being 35 μm in diameter. The spores are globose to elongate, yellowish sterile cells which have smooth wall. They are smaller in size (15–28 μm) than normal teliospores. Scanning electron microscopy of teliospore shows three distinct layers: the perisporium (sheath), the episporium and the endosporium. The perisporium is a fragile, fractured structure and the episporium is reticulated having numerous curved projections with blunt margins, due to which the surface of the spore looks rough. With the advancing maturity the perisporium ruptures at a few places and projections become visible. The surface projections are 3.1–4.0 μm thick in mature teliospore. An individual projection is composed of two double strands which are near the apex. The endosporium is thick and lamellate structure (Khanna et al. 1968; Gardener et al. 1983; Aggarwal et al. 1998).

Fresh teliospores have a period of dormancy and dormancy period of 1–6 months is needed prior to spore germination (Prescott 1984). The highest germination occurs with year-old teliospores (Mathur and Ram 1963; Bansal et al. 1983). Presoaking of teliospores for 4 days enhances the germination. Soaking of the spores in tap water, farm yard manure extract, soil extract, wheat straw extract and certain chemicals is reported to influence dormancy and the germinability of teliospores (Holton 1949; Mathur and Ram 1963; Rai and Singh 1979; Gupta and Singh 1983; Krishna and Singh 1983; Dhiman and Bedi 1984). Exposure of spores to a temperature of −196 °C in liquid nitrogen for 15 min also provides higher germination (Bansal et al. 1984).

The germination of teliospores is sensitive to temperature and light conditions. Munjal (1971) obtained maximum germination at 20 °C with pH 6.0 while Krishna and Singh (1982) found that spores germinate significantly at 20–25 °C under alternate light and darkness at pH 4.9. Zhang et al. (1984), however, found that optimal germination was at 15–22 °C, extremely low at 2 °C and no germination after prolonged exposure at 35 °C in darkness. According to Smilanick et al. (1985) highest germination of teliospores is obtained after 3 weeks incubation at 15–20 °C under continous light at pH 6.0–9.5. Aujla et al. (1986) noted that fresh spores put in plain water and kept in darkness provide 50% germination.

On germination, teliospore produces a stout promycelium measuring 10–190 μm length and 6–13 μm breadth. The promycelium may branch but only one branch bears a whorl of 60–185 primary sporidia at the tip (Mitra 1931; Krishna and Singh 1981). The primary sporidia are sickled shaped and have mean length and width ranging from 64.4 to 78.8 μm and 1.6–1.8 μm, respectively (Peterson et al. 1984). When teliospores germinate, the single diploid nucleus undergoes meiosis. Subsequent and rapid mitoses give rise to a large number of haploid nuclei which migrate from hypobasidium into the promycelium and primary sporidia, each of which receives one nucleus. The sensitive, short lived, mononucleate primary sporidia germinate terminally or laterally in free water, giving rise to thick mat of hyphae. In general, the colonies of the fungus are brittle, crustaceous, umbonate with wavy margins. Subsequently, from a cushion-like structure, two types of secondary sporidia, i.e. falcate (allantoid) and filiform sporidia, are produced. Most secondary sporidia are mononucleate. Mycelial cells that originate from either type of secondary sporidia are banana shaped, 11.9–13 μm long and 2–2.03 μm wide, and are forcibly discharged. These spores are the only infective entities. The filiform sporidia serve as the reproductive bodies to raise allantoid sporidia in successive generations (Dhaliwal and Singh 1989).

T. indica is a heterothallic fungus with bipolar incompatibility, controlled by multiple alleles at one locus (Duran and Cromarty 1977; Krishna and Singh 1983). The heterothallism demands fusion between compatible secondary sporidia because monokaryotic sporidia originating from germinated teliospores are individually non-infective. Therefore, dikaryotization between compatible types is a prerequisite. Bonde et al. (1997) suggested that dikaryotization occurs prior to penetration on glume surface. However, Sharma et al. (2008) have observed that the dikaryotization takes place inside the host tissue.

Physiologic forms of *T. indica* are known to occur in India. Mitra (1935) reported two forms of the pathogen based on spore size, but these variations were subsequently shown to be due to environmental effect (Mundkur 1943). In agreement with Mundkur's observation, Bansal et al. (1984) also confirmed that size of the teliospores can not be taken as a criterion for differentiating physiological races of *T. indica.* Aujla et al. (1987) differentiated four pathotypes K1, K2, K3 and K4 from different regions of Punjab and Himachal Pradesh on the basis of host pathogen interactions on 17 differentials. However, Singh et al. (1991) felt that race or pathotype concept in *T. indica* is not valid. They argued that in each annual cycle of the pathogen, nuclear fusion takes place between heterothallic secondary sporidia so the pathogenic isolate is a population, not a race. It is appropriate to classify the variation in isolates as aggressiveness. Keeping this argument in view, five aggressive isolates designated as KBAg-1, KBAg-2, KBAg-3, KBAg-4 and KBAg-5 have been identified (Singh et al. 1995). Still the issue of physiologic specialization or existence of races in *T. indica* is unresolved.

Examination of cross section of infected grains demonstrates that hyphae of the pathogen profilerate in the space formed by the disintegration of middle layer of parenchyma in the pericarp where they produce teliospore. In this process of proliferation, the nuceller projections get ruptured and hamper the flow of nutrients to

pericarp. The consequence is atrophy of seed and due to disruption of normal flow of nutrients it starves first the endosperm and then the embryo. The endosperm is shrunken and the space thus created gets filled with teliospores of the pathogen. The embryo is free from infection except under very severe infection (Cashion and Luttriell 1988; Aggarwal et al. 1994).

Roberson and Luttrell (1987) have studied the ultrastructure of teliospore ontogeny and found that teliospore of *T. indica* arise directly from a thin hymenial layer of hyphae. Teliospores appear as small, smooth walled circular bodies with 9 μm size at 10 days after inoculation. The size of the developing spores starts increasing at 15 DAI but the spore wall still remains smooth. The surface of teliospores shows small protuberances having round ends at 20 DAI and the size of the spore at this stage is around 25 μm. The rodlets become prominent at 25 DAI showing further increase in spore size from 34 to 40 μm. The surface ornamentation are fully developed at 28 DAI (Aggarwal et al. 1999).

16.3.1.3 Disease Cycle

The pathogen survives in the form of teliospores which fall in the soil during harvesting of the crop. The spores adhere to the surface of healthy seed and act as contaminant. The spores on the seed create problem only when they are transmitted through the seed and deposited on the soil surface where infection occurs. Obviously, soil-borne inoculum is the primary source of annual recurrence of the disease. Nagarajan et al. (1997) considered soil as the bank of teliospores of the pathogen. Normally, the resting spores buried in the soil germinate from the middle of February to middle of March when soil temperature and moisture are suitable. The germinated spores produce a germtube (promycelium) to come up to the soil level bearing tuft of 110–185 sickle-shaped primary sporidia. While still on the germ tube, the primary sporidia produce two types of secondary sporidia, that is, sickle-shaped or filiform-like primary sporidia and banana-shaped or falcate-type allantoid sporidia (Dhaliwal and Singh 1988). The filiform sporidia serve as the reproductive bodies to raise allantoid sporidia in successive generations. These spores are the only form that infects the wheat ear head. Allantoid sporidia are released forcibly (Dhaliwal and Singh 1988, 1989) and are carried by wind or rain splash to get deposited on the lower leaves of the host (Prescott 1986). They germinate in the presence of leaf wetness and produce secondary crop of spores on wheat and gramineous hosts. As the leaf surface dries, these spores get dispersed to upper leaves of wheat plant. Having reached the higher leaves through monkey jumps, they settle on the flag leaf during ear head stage. Occasionally, the air-borne allantoid sporidia get lodged on spike at anthesis and germinate on the glume surface, and the fungus becomes partially systemic in rachis and the rachilla. Subsequently, the hyphae spread to the adjacent florets and spikelets around the infection site. Hyphae of the fungus then grow through the base of glume into sub-ovarian tissue and enter the pericarp through the funiculus (Goates 1988). Further growth of hyphae is entirely within the pericarp. These hyphae become sporogenous produce teliospores in wheat seed to cause Karnal bunt infection in the next crop season.

Since Karnal bunt spreads through soil, seed and air, the disease development is influenced by a number of factors. The spores remain viable for a number of years in the soil lying 15 and 25 cm deep (Dhiman and Bedi 1989). According to Nagarajan et al. (1997), the soil acts as a bank and soil-borne teliospores have dormancy and retain their viability for more than 18 months when buried in soil at a depth of 5 cm. Teliospores buried very deeply in soil lose their viability due to low availability of oxygen and moisture. Moreover, microbial disintegration also affects the germinability of the spores (Rattan and Aujla 1990; Sidhartha 1992). It has been recorded that high level of inoculum in the soil is positively correlated with severe disease incidence at disease hot spots like Gurdaspur in Punjab (Singh and Srivastava 1992).

The spores on soil surface germinate from the middle of February to middle of March, if soil remains moist for a period of 7–10 days (Aujla et al. 1990). Krishna and Singh (1982) recorded that for teliospore germination, the optimum temperature range is between 20 and 25 °C. Sporidia formed after germination become air-borne. Hyphae that originate from primary or secondary sporidia produce large number of allantoid sporidia and comparative fewer filiform sporidia (Fuentes-Davila 1984). Filiform sporidia are more or less ineffective and cause very less infection (Warham and Burnett 1990; Sidhartha 1992). Since there is rapid vegetative multiplication of allantoid spores, the probability of successful fusion of opposite mating types increases, resulting in severe infection (Nagarajan et al. 1997).

The awn emergence stage corresponding to Z49-52 (Zadok et al. 1974) is the most vulnerable stage for establishment of Karnal bunt. Nagarajan (1991) hypothesized that at this stage, the flag leaf intercepts the allantoid sporidia and facilitate the run down of water droplets with sporidia. The aerobiology of the pathogen reveals that maximum sporidia in air are trapped at 30 cm and minimum at 90 cm, suggesting the possibility of spatial spread above the crop canopy by wind current. Hence, it appears that after release from the germinating teliospores, the sporidia first rest and multiply on lower leaves of the host plant and then move upward. Kumar and Nagarajan (1998) later, confirmed that the flag leaf provides base for allantoid multiplication and inoculumn build up. Diurnal periodicity in the sporidia was shown by Bains and Dhaliwal (1989). Spore trapping indicates that maximum sporidia are produced in the early morning hours between 2 and 6 a.m. (Sidhartha et al. 1995).

The establishment of the pathogen is highly dependent on favourable weather conditions. The prevalence of lower maximum (19–23 °C) and higher minimum (8–10 °C) temp. followed by high relative humidity and intermittent rains lead to well established infection of Karnal bunt (Joshi et al. 1981). These meteorological conditions prevail in the North-western region but are absent in the central India, thereby restricting the development of Karnal bunt in Madhya Pradesh and Rajasthan (Singh 2005). On the other hand, the mid and higher altitudes of Himalayas, where snow fall occurs every year, Karnal bunt can not establish and survive. In this region, the snowing and thawing reduce the viability of spores in soil. Possibly snowing induces cold dormancy and therefore, the chilled spores require more energy to germinate (Sidhartha et al. 1995). These spells of snow can delay the process of sporidial proliferation and ultimately teliospore germination; and result in disease

escape. According to Singh and Srivastava (1997) the chances of survival of teliospores are more under the irrigated conditions of rice-wheat cropping system in North-western region. They emphasised that the spores surviving in rice field are brought to the soil surface from different depths and serve as one of the sources of inoculum for fresh infection of Karnal bunt during wheat season.

16.3.1.4 Management

The control of Karnal Bunt is difficult due to its mode of perpetuation through soil and seed. Hence, an integrated approach for tackling the disease is considered most ideal. Genetic studies reveal that Karnal Bunt resistance is polygenic and partially dominant (Gill et al. 1993). Bag et al. (1999) have shown that resistance in wheat genotypes HD 29, HP 1531 and W 485 is conditioned by a single recessive gene and all the three accessions carry a non-allelic gene for resistance. Nanda et al. (1995) has also observed that dominant alleles are involved with resistance. Resistance to Karnal bunt is governed by two genes in genotypes Luan, Allila, Vee 7/ Bow, Star, Weaver, Milan, Sasia and Taracio/ Chil, and monogenic resistance in Cettia, Irena, Turacio, Opata, Picus and Yaco. Singh et al. (1995) found that digenic genotypes possess higher level of Karnal bunt resistance than those lines with a single gene. Sharma et al. (2005, 2007) found that HD 29, W 485 and ALDAN 'S'/ IAS 58 each carry two resistance genes, whereas 3 genes are involved in H 567. 71/3 PAR. Genetic analysis shows that HD 2329 when used as the recurrent parent in backcross programme, gives more resistant progenies. Monosomic analysis using three resistant lines HD 29, WL 6975 and WL 2328 and the susceptible WL 711 indicates that the gene governing resistance to Karnal bunt is located on chromosomes 1D, 2D, 3B, 3D, 5B and 7A. The backcross involving some allien sources and cultivar Kalyansona yielded some Karnal bunt resistant derivatives (Sawhney and Sharma 1996). Sirari et al. (2008) proposed use of mixture of most virulent isolates to identify Karnal bunt resistance genes.

Wild species of wheat are considered as valuable source of Karnal bunt resistance. Some synthesized amphiploids involving Karnal bunt resistant accessions of *Triticum monococcum, T. boeticum* and *Aegilops squarrosa* were free from Karnal bunt, indicating that the resistance is expressed in the presence of *durum* complement (Multani et al. 1988). Some accessions like *Triticum tauschii, T. dicoccoides, T. spelta album, T. spelta grey, T. durum/Agropyron elongatum,* Chinese spring /*A. elongatum, Chinese junceum* and Chinese spring accessions have been found to be immune to Karnal bunt (Tomar et al. 1991). *Triticum araraticum* (wild form of *Triticum timopheevi*, 2n = 4× = 28 AAGG) is reported to be a useful source of *Tilletia* species and to a number of other wheat pathogens (Bijral and Sharma 1995).

A large collection of wheat germplasm under field inoculated condition is being tested using standardized techniques and a numerical rating system (Aujla et al. 1980). Currently popular wheat cultivars like PBW 502, HS 365, PBW 34, HP 1731, NW 1014, Raj 1555, HD 4672, WH 365, etc. possess fairly good degree of disease resistance. Consequently, the extent of Karnal bunt damage has reduced in recent years.

Crop rotation following non-cultivation of wheat crop for two consecutive years as an agronomic adjustment has been advocated by Mitra (1937) and Padwick (1939). Since the higher dose of nitrogenous fertilizers increases susceptibility of plants, adjustment of water and fertilizers in disease affected areas is recommended (Bedi et al. 1949; Singh and Singh 1985). Biological mulching, such as inter-cropping wheat with chickpea and covering the inter row space with transparent polythene, reduces the disease severity. Avoiding early sowing of wheat also results in poor incidence of Karnal bunt. Zero-tillage has shown a reduced incidence of Karnal bunt in comparison to furrow raised bed (FIRBS) and conventional tillage system. If zero tillage is followed for a few years, it may help in reducing the effective soil inoculum and thereby reducing the disease incidence over times (Sharma et al. 2007).

In North-west India, a variety of crop sequences are followed and field gets vacated at different times. The differing planting time delays wheat sowing and staggers the heading emergence stage and thus creates a temporal hurdle for *T. indica.* Coincidence of winter rainy days and ideal temperature conditions do not prevail over such a long heading period.

Seed dressing fungicides such as aureofungin, ethyl-mercury chloride, ethyl-mercury phosphate, fentin acetate, triphenyltin chloride, fentin hydroxide, indar, benomyl, carbendazim thiuram disulphide etc. have been found to restrict seed-borne inoculum of the pathogen but these chemicals have little effect on soil-borne teliospores. Foliar sprays with systemic fungicides give effective control of the air-borne allantoids sporidia on wheat foliage. Among several fungicides evaluated for spray application, propiconazole (Tilt 250 EC) at the heading stage provides 71.4–100% disease control (Aujla et al. 1989; Singh et al. 1989). No residues of propiconazole were detected in wheat grain or straw when sprayed at 250 ml/ha (Singh et al. 1991; Sharma et al. 1994). Some other fungicides such as bitertanol (baycor), tebuconazole (folicur) and cyproconazole (SAN 619F) have also potential for commercial exploitation at times of dire need (Singh et al. 1985; Singh and Srivastava 1992).

Antagonistic potential of *Trichoderma viride, T. harzianum* and *Gliocladium deliquescens* on germination of teliospores of *N. Indica* was observed by Aggarwal et al. (1996). Amer (1995) also evaluated some fungal and bacterial microorganisms and recorded that bio-control agent *T. lignorum* causes significant decline in teliospore germination when used as soil inoculant under field and glasshouse conditions. Scanning electron microscopic investigation demonstrated over growth of *T. lignorum* on mycelium of *N. Indica.* Ultra-structural observations showed that the antagonist mediated host mycelium by penetration pegs and resulted in disintegration of the host mycelium.

Since Karnal bunt is prevalent only in a few countries around the world, there is general quarantine imposed against the disease. Most countries insist on a zero tolerance on shipment of wheat. In Mexico there is a domestic quarantine restricting the movement of wheat material from one area to another. European and Mediterranean Plant Protection Organisation (EPPO) has presented a list of A-1 quarantine pests including *T. indica*. In China, wheat consignment is accepted from

other countries only after the wheat lots are declared as having zero infection. USA has declared *T. indica* as a quarantine pest after introduction of Karnal bunt in Arizona, Texas and New Mexico. Canada has maintained a rigid position on the disease and has imposed zero tolerance (Singh 2005).

In India, there is no domestic quarantine. However, seed certification is desired for production of disease-free seed, especially in seed multiplication programme. The National Seed Project lays down a maximum of 0.05% and 0.25% level of Karnal bunt infection for foundation and certified seeds, respectively (Agarwal and Verma 1983). Washing test is an efficient method for quarantifying the externally seed-borne spores in seed lots. If teliospores contamination is above 25 spores/gram, seed lot is rated as contaminated (Agarwal et al. 1973).

Seed contamination with Karnal bunt spores is possible at the time of threshing of wheat crop. Smoke caused by burning of wheat stubble /straw in the field, combine and threshing machines, transportation by trucks and railway wagons are reported to promote spread of teliospores over long distance (Bonde et al. 1987). Therefore, as part of the Karnal bunt risk management one must desist from setting fire to standing crop stubble and threshing machine/grain wagon should be thoroughly cleaned (Malik and Mathre 1998).

The FAO and other global organisations are of the view that quarantine should be based on risk analysis using appropriate testing procedures (Singh 2005). Nagarajan (1991) has developed a computer-based pest risk analysis model which can be put for analysing the possibility of Karnal bunt occurrence in any geographic situation. The GEOKB is suitable for assigning probability value of disease occurrence at locations in different latitudes and agronomic conditions of wheat growing there and the second part of the software KBRISK takes care of climatic data to evaluate probability of Karnal bunt occurrence or establishment. Scientists and regulators are prospective clients for these PRA models.

16.3.2 *Fusarium* Head Blight

Fusarium head blight (FHB) caused by *Fusarium* spp. is a devastating disease responsible for extensive yield and quality losses in wheat in humid and semi humid regions of the world (McMullen et al. 1997). *Fusarium* spp. a complex group of fungi with ascomycete teleomorphs genus includes various pathogenic species which may cause serious diseases in wide range of economically important plants and can produce noxious secondary metabolites which are responsible for some human and animal diseases (Marasas et al. 1984). *Fusarium* head scab of wheat was first reported in England around 1884; however scab is generally more important in warm and humid areas. Scab can cause significant yield and quality losses as well as toxicoses in humans and animals. *Fusarium* head blight (FHB) or *Fusarium* ear blight or head scab of wheat caused by *Fusarium* spp., is an economically devastating disease of wheat that reached epidemic proportions in the United States during the 1990s, resulting in losses of approximately three billion dollars to US agriculture due to poor seed quality and low yields (Windels 2000). FHB of wheat is a

global problem with recent outbreaks reported in Canada, Europe, Asia, Australia and South America (McMullen et al. 1997). Epidemics in China are most common and losses upto 2.5 million tons of wheat grains have been recorded in epidemic years. Concerns about toxin-related illnesses caused by *Fusarium* in animals and humans have increased in recent years. The disease has emerged as one of the most important plant diseases worldwide in the last century.

Currently, FHB is of minor importance to India but can cause significant yield loss if rain occurs during mid anthesis in the foot hills of Punjab, Himachal Pradesh and Tamil Nadu. Changes in tillage practices, principally the move towards conservation tillage and reduced-till systems, contributed to the recent FHB epidemics in Upper Midwest of the United States (Dill-Macky and Jones 2000). The *Fusarium* diseases which have already established in the Indian sub-continent are likely to increase under the impact of global warming and the fast spread of reduced tillage practices in the main wheat belt, i.e. the northwest plains of India. Keeping in view the global climate change, systematic research work on FHB was initiated at Directorate of Wheat Research, Karnal in 2000 under Indo-Swiss Collaboration in Biotechnology (ISCB) project. Evaluation of material for resistance to FHB was carried out under AICW&BIP. In India, during *Kharif* 1984, disease was observed on high yielding wheat varieties in Nilgiris (Brahma and Singh 1985). Disease was also reported from Arunachal Pradesh (Roy 1974), Punjab (Chahal et al. 1993; Singh and Aujla 1994; Saharan et al. 2004). In Punjab, FHB sometime becomes an important constraint to wheat production (Singh and Aujla 1994). In April-May, 1990, a severe outbreak of this disease was observed in some affected areas in Amritsar and Gurdaspur districts of Punjab and variety HD 2329 was badly affected (Chahal et al. 1993). During 2005, frequent rainfall during flowering favoured disease development and caused enormous losses in yield and quality in Gurdaspur district. During a survey conducted on March 29–30, 2005 in the North Western Plain Zone, moderate to high incidence of FHB was observed in the bread wheat cv. PBW 343 (10–50% infected heads) and durum wheat cv. PDW 274 (>90% infected heads) in the Gurdaspur district (Bagga and Saharan 2005; Saharan et al. 2007).

16.3.2.1 Symptoms

Initial infections appear as small, water-soaked spots at the base or middle of the glume, or on the rachis. Water soaking and discoloration then spreads in all directions from the point of infection. A salmon-pink fungal growth may be seen along the edge of the glumes or at the base of the spikelet. Infected grains become shrivelled with a floury discoloured interior. Premature death or bleaching of spikelets is also a common symptom and can be seen on emerged immature heads where one or more spikelets or the entire head may be affected. In severe infection, the peduncle may turn dark brown. Inoculation of wheat heads with macroconidia of *F. graminearum* with both syringe inoculation and cotton web technique developed symptoms as white/pink fungal growth (Saharan et al. 2004).

16.3.2.2 Pathogens

Fusarium head blight has been associated with up to 17 causal organisms, of which *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein] Petch) is the principal pathogen responsible for head blight in many countries. Other related species such as *F. culmorum* (Smith) Sacc., *F. avenaceum* (Fries) Sacc., *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* J. Sheld), *F. oxysporum* Schlect., *F. poae* (Peck) Wollenw and *Microdochium nivale* (Fries) Samuel & Hallett may contribute to the head blight complex but are generally less important than *F. graminearum* (Wiese 1987). In India, *F. avenaceum* and *F. graminearum* causing ear blight and scab of wheat on such varieties as Sonalika, Kalyan Sona, Safed Lerma and Lerma Rojo were first reported by Roy (1974) in samples received from Siang District of Arunachal Pradesh. *Fusarium graminearum* causing head scab of wheat was also observed by Brahma and Singh (1985) on varieties viz., Sonalika, Agra Local, HW 517, HW 741, HW 1042, E 9382, E 2670 and C 306 at IARI, Regional Station, Wellington. Several *Fusarium* spp. (*M. nivale*, *F. compactum* (Wollenw) Gordon, *F. verticillioides*, *F. subglutinans*, *F. oxysporum* and *F. pallidoroseum* (Cke) Sacc. were also found associated with head scab complex in the Gurdaspur area of Punjab (Singh and Aujla 1994). In addition to these, *F. semitectum*, *F. compactum* have also been associated with head scab of wheat (Mann and Nanda 1999; Kaur et al. 1999).

An extensive survey was carried out by DWR (now IIWBR) for collection of head scab samples since 2000–2010 in the mid and high Himalayas of Himachal Pradesh (Lahaul valley, 12, 000 ft. amsl), Wellington (Tamil Nadu) and Punjab. Six *Fusarium* species viz., *F. graminearum*, *F. verticillioides*, *F. oxysporum*, *F. equiseti*, *F. solani* and *F. semitectum* were isolated from head scab infected samples. *F. graminearum* was found in most of the samples collected from Lahaul valley, Punjab as well as from Wellington. All *Fusarium* species were inoculated on susceptible var. PBW-222 and Koch's postulates were proved. In India, three *Fusarium* spp. viz., *F. graminearum*, *F. verticillioides* and *F. oxysporum* are mainly responsible for FHB (Saharan et al. 2003). *Fusarium graminearum* is the predominant causal agent of head blight of small grain cereals in the United States, Europe and India (O'Donnell et al. 2000; Saharan et al. 2003; Zeller et al. 2004). *F. graminearum* Schwabe, a haploid ascomycetous fungus and the major causal agent of FHB disease of small grain cereals, has received considerable attention by the scientific community. Due to this international interest, *F. graminearum* was identified as a priority for whole-genome sequencing by the Broad Institute Fungal Genome Initiative and in spring 2003 became the second plant-pathogenic fungus for which the whole genome sequence has been made publicly available.

16.3.2.3 Disease Cycle

Establishment of infection in the spike whose mother plant provided with cotton platform for placing inoculum implied the capability of spores to move upwards from ground level. In case of HB, spores could reach I leaf within 48 h after their placement on cotton platform. It took 72 h for spores to reach II leaf while 96 h to reach III leaf. Spores landed on boot leaf only after 120 h of their placement on cotton platform. In

case of KB, spores witnessed a movement of comparatively slower pace. Only few spores had reached first leaf within 48 h. Their significantly sizeable quantities reached first leaf only after 72 h of placement on cotton platform. The second and third leaves were reached respectively after 96 and 120 h. The boot leaf could also be accessed after 120 h. Kaur et al. (2007) reported high humidity for 48 h immediately after inoculation conducive for the proper development of disease.

16.3.2.4 Management

Preliminary work on the feasibility of using microorganisms to reduce FHB has been initiated at Directorate of Wheat Research (now IIWBR), Karnal. Effect of bioagents on radial growth of *Fusarium semitectum* and *F. graminearum* isolates was studied in vitro (Saharan et al. 2008). Bioagents viz., one isolate of *Gliocladium virens*, two isolates of *Trichoderma harzianum*, three isolates of *T. viride* and one isolate of *Pseudomonas fluorescens* were evaluated in vitro to see their effect on inhibition of mycelial growth of *F. semitectum* and *F. graminearum* isolates. After 120 h of incubation, there was significant reduction in mycelial growth of *F. graminearum* isolates of Wellington, Nilgiri Hills, Tamil Nadu (W3, W5, W11 & W18) as compared to control with all bioagents. *T. viride*-M resulted in maximum inhibition of 52.00% in mycelial growth of *F. graminearum* isolate of Wellington (W18) as compared to control. Among *F. semitectum* isolates, maximum significant inhibition of mycelial growth was recorded in isolate DBN2 with *T. viride* 5-2. During 2004–05, seed treatment with *P. fluorescens* (Biomonas) and *T. viride* (Bioderma) reduced the spike infection (46.86%) as compared to control (untreated check) in var. UP 2338. Significant reduction in spikelet infection with all the bioagents as compared to control (untreated check) was also observed during 2005–2006 in same cultivar. Seed treated with *T. viride* 5-2 resulted in maximum disease control of 59.62% in variety UP 2338 followed by *T. harzianum*-1 (Hisar isolate) as compared to control. Seed treatment with *P. fluorescens* reduced disease severity significantly (45.66%) in variety, UP 2338 as compared to control (untreated check). Antagonist (seed treatment with *P. fluorescens*) reduced disease severity (% spikelet infection) by as much as 40.70% in variety, WH 542, while antagonist, *T. harzianum* (Hisar isolate) reduced disease severity by 34.98% compared with control (untreated check). Biocontrol may provide an alternative form of protection against FHB when chemical applications at heading raise concerns over chemical residues in the harvested grain. The results represent the first demonstration of the feasibility of biological control of FHB on two popular cultivars of wheat in India which holds considerable promise as FHB control with bioagents is more amenable than other foliar diseases because the period of vulnerability to severe infection is quite short, usually confined to less than 2 weeks.

Bagga (2007) evaluated the efficacy of fungicides viz., Tilt, Score, Folicur, Topas, Amistar, Bayleton and Kavach as foliar spray for managing FHB under natural infection at PAU Agricultural Research Station, Gurdaspur. Environmental conditions were very favourable for FHB development. All above seven fungicides reduced FHB incidence by 5.2–74.4% and severity by 22.40–89.30%, Amistar (azoxystrobin) followed by triazole fungicides Folicur and Topas gave maximum significant disease control as compared to unsprayed control in cv.

PBW 502. Application of Tilt 25 EC, another triazole fungicide was found least effective in reducing FHB but it showed good efficacy against FHB in previous studies (Bagga et al. 1997).

Most wheat cultivars currently grown in India are susceptible to FHB. Control of the disease has been difficult, because of the complex nature of the host/pathogen/environment interaction. Promising options for controlling FHB include chemical measures and the development of resistant cultivars. Fungicides like tebuconazole, prochloraz and bromuconazole are effective for controlling FHB but food safety concerns and inconsistent results due to the complexity of causal organisms, timing of application and development of fungicide resistance in the pathogen population, limits the chemical management option. Thus, growing of wheat cultivars resistant to *Fusarium* spp. has been accepted as the most reliable, economic, environment friendly and effective method of managing this disease.

Methods of inoculation for creation of head scab epiphytotics in poly house conditions has been standardized and around 2000 Advance varietal trial material, RILs, CIMMYT material have been evaluated and resistant sources have been identified. Seventy five wheat genotypes of 9th SRSN were artificially inoculated with *Fusarium graminearum* with cotton web technique in 2005–06. None of the synthetic line was found resistant to head scab. Out of 39 genotypes of 10th nursery, four genotypes, SUM3/3/CS/LE.RA//CS/4/YANGMAI 158, GONDO, TUI/MILAN and PEL 73007 were found moderately resistant to head scab pathogen (*F. graminearum*) in 2006–07. Out of 47 genotypes of 11th nursery, only one entry (FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//…) was found moderately susceptible to head scab in 2008–09. During 2009–10, out of 42 genotypes of 12th nursery, six were found completely free from head scab. During 2000–01 to 2009–10, 1206 advance wheat lines including popular cultivars were also screened and resistance sources have been identified (Kumar et al. 2001, 2002a, b; Saharan et al. 2003, 2004; Sharma et al. 2010).

16.3.3 Spot Blotch Disease

The disease is predominant in south east Asia and in the Indian subcontinent. In India, the disease was first recorded in Bihar as early as 1914 by Mohy (HCIO No. 12508). The disease frequently occurs in warm and humid areas of Bihar, West Bengal, Uttar Pradesh, Orissa, Assam, Madhya Pradesh, Maharashtra and Karnataka. It had been a serious problem basically in north eastern region of the country, but due to climate change and crop intensification this disease has made its appearance on large scale in north western, peninsular region and central zones of the country. The pathogen is also present in China, Australia, Brazil, North and Latin America, Canada and parts of Europe (Kumar et al. 2002b).

During the last more than three decades there has been a remarkable progress in breeding for rust resistance which has prevented rust epidemics by planned usage of vertical genes in different epidemiological zones in the country. These factors have encouraged population build up of minor diseases like spot blotch (Singh and Srivastava 1997). Therefore, spot blotch caused by *Bipolaris sorokiniana* ((Sacc.)

Subram. & Jain) also known as *Helminthosporium* leaf blight or foliar blight has emerged as one of the most important diseases limiting wheat production in warmer, non-traditional growing areas causing up to 36% loss under favourable conditions. The disease is worldwidely distributed and is important in Africa, South America, Australia, Canada and Asia and particularly to Indian sub-continent having warm and humid environments (Singh and Srivastava 1997). Recently, rice and wheat rotations have occupied larger areas in Punjab, Haryana, western U.P. and late sowing of wheat is a common practice. The delayed sowing experiences a warm and humid weather in February which is favourable for spot blotch. Moreover, climate change particularly sudden rise in temperature in the month of February with intermittent rainfall has also created favourable environment for disease in the areas where disease was not known to occur. In 1990–1991, the disease appeared in an epidemic form in some districts of western Uttar Pradesh on wheat varieties HD 2329 and HD 2285, mainly due to late sowing and warm humid environment in March (Singh et al. 1993). Since then, this disease has emerged as a major problem in North-western as well as in the Peninsular India and has acquired the status of national importance instead of regional one (Sharma et al. 1998). Development of cultivars resistant to spot blotch is not an easy task as the resistance to spot blotch found in the germplasm is not satisfactory and none of the commercial cultivars show resistance.

The importance of spot blotch is expressed in terms of losses which are variable but are very significant. Yield losses due to this disease are important in fields with low inputs and under late-sown conditions. In India, there are reports indicating that losses are variety dependent. Nema and Joshi (1971) reported that at flag leaf stage in Sonora 64, the loss incurred was hardly 3%, while S-227 showing maximum susceptibility suffered losses of about 20%. A multi-location trial on yield losses due to foliar blight indicated 2.7–36.2% loss in yield and 0.1–16.3% reduction in grain weight at Pantnagar, Kanpur and Faizabad. Under natural conditions, losses as high as 20% and 22% in the wheat varieties UP 262 and HP 1633 can occur, respectively. In eastern Indian sub-continent, on farm studies indicated crop losses up to 16% in Nepal and 15% in Bangladesh (Duveiller and Garcia Altamirano 2000). Losses of 85% were assessed in Zambia (Raemaekers 1988), 40% in the Philippines (Lapis 1985) and 30% yield loss in Netherlands (De Miliano and Zadoks 1985). During favourable years, losses from spot blotch alone were between 30% and 86% in Brazil and in some fields reached to 100% (Mehta 1985). Hetzler et al. (1991) reported that yield losses due to this disease were estimated to be up to 87% in highly susceptible varieties. This spot blotch disease in complex form with tan spot (*Pyrenophora tritici repentis*) is a major constraint to wheat production in South Asia causing an average of 30% loss and has emerged as most significant disease (Joshi et al. 2007).

The average yield losses due to combined effects of leaf blight pathogens for South Asia and India are reported to be 19.6 and 15.5%, respectively. The yield losses ranging from 6.3% to 50.6% have been reported from foliar blights influenced by cultivars and agro-climatic zones (Singh et al. 2004). Due to widespread losses, this disease is considered as the most significant disease of wheat not only in north-eastern plain zone of India (Saari 1998) but in all wheat-growing regions (Joshi et al. 2007).

Fig. 16.6 Spot blotch disease of wheat

16.3.3.1 Symptoms

The spot blotch pathogen is capable of producing disease symptoms in all plant parts, i.e. internodes, stem, nodes, leaves, awn, glumes and seed. The pathogen causes pre- and post-emergence damping off, seedling blight, foot rot, leaf spot and spike blight in different stages of growth of the plant. Early lesions on the leaves are characterised by small, dark brown lesions 1–2 mm long without chlorotic margin (Fig. 16.6). In susceptible genotypes, these lesions extend very quickly in oval to elongated blotches, light brown to dark brown in colour giving a blighted look. Root and crown infections in severe form may lead to complete drying of infected plants without seed production (Zillinsky 1983). Under favourable conditions, spikelets may be affected causing grain shriveling and black point.

16.3.3.2 Pathogen

Bipolaris sorokiniana (Sacc.) Shoem. (Syn. = *Drechslera sorokiniana* (Sacc.) Subrm. & Jain; *Helminthosporium sativum* P.K. & B.) is a dematacеous, hyphomycetous fungus. The perfect stage of the pathogen has been identified as *Cochliobolus sativus* (Ito & Kurib) Drechsler ex. Dastur. The pathogen develops olivaceous brown to black colonies on culture media. A detailed description of *Bipolaris sorokiniana* (Sacc) Shoemaker can be obtained from CMI's Sivanesan and Holliday (1981). In the older literature, several synonyms of the anamorph have been used, i.e. *Helminthosporium sorokinianum, Drechslera sorokiniana* and *Helminthosporium sativum* (Maraite et al. 1998). Shoemaker (1959) proposed the generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight or curved conidia germinating by one germ tube each end. The former genus *Helminthosporium* was divided into three

anamorphic genera: *Bipolaris*, *Drechslera* and *Exherohilum* with the teliomorphic stages *Cochliobolus, Pyrenophora* and *Setosphaeria,* respectively. *Bipolaris sorokiniana* is characterized by thick walled, elliptical conidia (60–120 µm × 12–20 µm) with five to nine cells. In axenic culture, the mycelium is composed of hyphae interwoven as loose cottony mass and appears white or light to dark grey depending on the isolates. The fungus is differentiated from other members of the *Bipolaris* genus on the basis of morphological features of conidiophores and conidia. Conidia appear black and shiny under light microscope and smooth walled with polar germ pores under scanning electron microscope (Aggarwal et al. 2002).

There are a few early reports indicating the presence of phytotoxic substances in culture filtrate of *B. sorokiniana* (Ludwig 1957; Gayad 1961). The most active and abundant phytotoxin produced by this pathogen is 'helminthosporol' ($C_{15}H_{22}O_2$), which is a hydrophobic sesquiterpene with restricted thermal stability and low water solubility. Aldehyde form of this toxin, prehelminthosporal causes an inhibitory effect on proton pumping by H^+-ATPase (Olbe et al. 1995). Production of compound 'sorokinianin' has also been reported which has inhibitory effect on seed germination (Nakajima et al. 1994). Recently, another new compound produced by this pathogen has been purified and characterized through NMR and GC-MS and has been identified to be 'Bipolaroxin', having molecular weight 264.5 g/mol which is a compound belonging to family Eremophilane. Toxin bipolaroxin is produced in the range of 0.05 µg/ml to 0.72 µg/ml culture filtrate by different isolates of *B. sorokiniana* as quantified through high-performance liquid chromatography (HPLC) (Aggarwal et al. 2008; Jahani et al. 2014). This is the first report on production of this toxin by *B. sorokiniana*. Bipolaroxin showed toxicity to *Phalaris minor*, *Avena sativa* and *Cynodon dactylon* but not to *Amaranthus tricolour.*

16.3.3.3 Disease Cycle

Most *Helminthsporium* species are favoured by moderate to warm temperatures (18–32 °C) and particularly by humid weather (Singh and Srivastava 1997). Spot blotch is probably the most serious leaf blight disease of wheat in the mega environment characterized by high temperature (coolest month greater than 17 °C) and high relative humidity. However, it has been increasingly recognized as a problem in optimum and irrigated conditions, also known as the ME 1 mega environment characterized by irrigated, low rainfall and temperate growing conditions (Van Ginkel and Rajaram 1993).

Infected seed, infected crop residues, volunteer plants, secondary hosts and free dormant conidia in the soil serve as source of *B. sorokiniana* inoculum. This hemibiotrophic fungus normally sporulates on the necrotic tissues throughout the growing season and reaches the ear heads, finally returning to seed, which ensures the most efficient mechanism for pathogen survival (Reis 1991). When such seeds are sown in the field, the coleoptile readily gets infected resulting in seedling infection, which provides inoculum for the growing crop. Mondal (2000) reported that infected seeds and soils infested either with conidial suspension or colonized grains may serve as potential sources for the survival of *B. sorokiniana* resulting in germination failure, seedling mortality and spot blotch development in wheat. However, according to Shaner (1981), infected seed appears to be the main source of inoculum. Soon

after sowing, the fungus starts growing on the moistened seed, and just after seedling emergence as early as the first leaf stage, sporulation is induced in the presence of direct sunlight (Spurr and Kiesling 1961). Conidia produced on the first leaves can be disseminated by rain splashes and wind, thus building up polycyclic epidemics. *B. sorokiniana* in addition to wheat has a large number of grasses as host that co-exist in an area, but their role in perpetuation of the disease in Indian subcontinent is still not clear (Nagarajan and Kumar 1998)

Bipolaris sorokiniana affects small grain cereals also (Zillinsky 1983). A wide variety of other grasses act as potential hosts (Nelson and Kline 1966). Lapis (1985) noted that various grasses and broad leaved weeds such as *Commelina diffusa, Chloris barbata, Dactylactenium aegypticum, Eleusine indica, Cyperus difformis, C. fibricatus, Imperata cylindrica, Cynadon dactylon, Paspalum conjugatum, Laptochloa chinensis, Rottboellia exallata, Brachiaria distachya, B. mutica* and *Echinocloa colonam,* grow year round in Philippines and harbour the pathogen. In India, *B. sorokiniana* has been found on *Phalaris minor* and *Launia splenifolia* (Singh et al. 1995).

16.3.3.4 Management

The complexity of this blight in the main wheat belt and of the conditions, in which it occurs, makes it essential to seek a holistic approach for management. The basic principle involved in the management of *B. sorokiniana* is reducing the inoculum source. Crop rotation with proper fertilization, clean cultivation besides resistance sources, fungicidal and biological control serve as IPM practices for lowering the disease levels. The disease severity can be reduced by low seed rate (100 kg/ha), wider space rows (23 cm), reduced NPK doses (60:30:30), low number of irrigations (3) and timely sowing (30th November) (Singh et al. 1998). Late planting of wheat should be avoided so that crop does not coincide with hot and humid period. Seed treatment with fungicides will help protect germinating seed and seedlings from pathogen causing seedling blight. Fungicide seed treatments include: Captan, mancozeb, maneb, thiram, pentachloronitrobenzene (PCNB), carboxin, iprodione and triadimefon (Stack and McMullen 1988; Mehta 1993). Fungicides such as triademinol, fentinaacetate, propiconazole, Dithane Z-78, Iprodione and Imazalil provide effective control of foliar phase of the disease. However, their use under environment conducive to high disease pressure is not economical.

Biocontrol strategy for the management of this disease has been explored. Mandal et al. (1999) tested the antagonistic behaviour of 16 different microorganisms and found that *Trichoderma reesei* and *Chaetomium globosum* significantly reduced the radial growth of *Bipolaris sorokiniana*. Culture filtrate of *Chaetomium globosum* effectively suppressed spot blotch disease in green house when sprayed at 1:1 dilution (Aggarwal 2004). A potential strain Cg-2 of this biocontrol agent has been identified, which showed inhibitory effect on germination of conidia and mycelial growth. Foliar spray with this biocontrol agent at 10^6 cfu/ml is effective in controlling the disease (Aggarwal et al. 2004). This isolate produced 13 secondary metabolites when extracted by solvent extraction method (Aggarwal et al. 2007), out of which five characterized through NMR and GC-MS had antifungal activity.

The most economical way to manage the disease is through resistance breeding. In India, resistance to *B. sorokiniana* is low among commercial wheat cultivars. Many varieties like HD 2329, HD 2285, Sonalika, HUW 234, K 8804, HS 284, UP 2338, UP 2003, C 306, WH 147, etc. are susceptible to this pathogen. Sources of resistance against *B. sorokiniana* have been identified by various workers under natural and artificial epiphytotic conditions. Two hundred and ninety one promising wheat genotypes were screened and out of these 10 genotypes namely, CB(BW)-351, CB(BW)-355, MRANG ALD/AN'S', MON 's'/ALD 's', UHU, BAU 4, K 9204, BW 14989, HW 2012 and HW 2014 were rated as resistant (Singh et al. 2005a). Performance of 16 commercial cultivars against *B. sorokiniana* was assessed under artificial conditions and only two genotypes viz., WH 157 and WH 283 were moderately resistant (Karwasra et al. 1998). Mexican varieties Alovdra, Cocoraine, Cugap; Chinese genotypes, Ning 8201, Longmai 10, Yangmai 6 and Brazilian cultivars, BH 1146, CNT 2, PAT 7219, Ocepar 7 have best resistance (Mehta 1985; Singh et al. 2007). Genes for resistance from several sources other than wheat have to be combined to achieve sufficient resistance. Dhaliwal et al. (1986) have found very little resistance to spot blotch in the germplasm of wild wheats and *Aegilops* species. While none of the *Triticum urantum* assessions was resistant, *Aegilops squarrosa*, other D genome species and some S genome *Aegilops* spp., except *A. speltoides*, possessed resistance. Transfer of resistance from such species to *T. aestivum* is rather complicated and difficult, mainly due to differences in levels of ploidy. Rajaram (1988) suggested an alternative approach of pyramiding genes from different sources. At CIMMYT, spot blotch resistance from *Agropyron distichum*, *A. curvifolium* and *Elymus gigantens* has been transferred to wheat (Kohli et al. 1991). The wheat wide cross hybrid with *A. curvifolium* (CS/AC 4) proved to be a good resistance source, and this alien germplasm could be exploited.

Inheritance studies on resistance to spot blotch are limited and the nature of inheritance is still debatable. Reports indicate both monogenic (Srivastava et al. 1971; Adlakha et al. 1984) and polygenic (Velazquez Cruz 1994) types of resistance. Singh et al. (2000) suggested that inheritance of spot blotch resistance is controlled by two pairs of complementary recessive genes based on their work on HP 1633, K 8962, UP 2338 cultivars. Earlier Singh et al. (1997) using NI 8289 x UP 262, CP-AN-1910 x UP 262 and NI 8289, HUW 234 (inter varietal resistance x susceptible) has also arrived at similar conclusions. Adlakha et al. (1984) noted that inheritance to spot blotch is simple but governed by one or two dominant factors. Srivastava (1982) dealing with seedling resistance also noted that resistance is governed by two complementary dominant genes. However, experience of wheat workers to achieve partial resistance in breeding population has suggested polygenic type of resistance (Dubin and Van Ginkel 1991). Duveiller and Gilchrist (1994), Dubin and Rajaram (1996) and Joshi et al. (2004a) suggested that around three additive genes are responsible for spot blotch resistance in wheat lines. Spot blotch resistance governed by quantitative trait loci (QTL's) have been mapped to chromosome 1S and 5S in barley in adult stage (Steffenson et al. 1996). One hundred and thirty-nine single-seed descent (SSD)-derived recombinant inbred lines (RILs, F_8) of the cross between Yangmai 6 (resistant) and Sonalika (susceptible) were investigated for QTLs. The distribution of 139 RILs suggested that spot blotch resistance is

polygenic. Earlier studies also suggested a polygenic control for spot blotch resistance (Joshi et al. 2004a).

The breeding efforts towards selection of resistant populations are marred by absence of suitable markers. Some efforts have been made in this direction. Plant height and days to maturity, known to influence the expression of spot blotch resistance (Dubin et al. 1998) were shown to have no genetic relationship with resistance (Joshi et al. 2002). Leaf tip necrosis, a phenotypic marker has been found to be associated with resistance to this pathogen, which would facilitate selection for resistance breeding (Joshi et al. 2004b). Earlier, waxy leaf surface, a physico-chemical marker has been shown to be associated with resistance in genotype, Pusa T3336 (Das et al. 1999). Further efforts are in progress to identify/develop molecular markers linked with resistance.

16.3.4 Loose Smut

Loose smut is an internally seed-borne disease, wherein the mycelium remains dormant in the embryo.

16.3.4.1 Symptoms

The symptoms of disease become visible after ear emergence. However, in some varieties like ‘sonalika’, the leaf shows pale discoloration even before ear emergence. A mass of dark, ovivaceous brown spores is seen in the infected ears in place of seeds (Fig. 16.7). Only the rachis is intact but it may be slightly shorter then the rachis of healthy tiller. The symptoms may develop on the flag leaf also in some varieties. All tillers on an infected plant can produce smutted heads and infected plants produce few or no grain. The black powdery spores are blown away by wind to leave a bare stalk or rachis. The spores are released as the rest of the crop is flowering. They infect the developing grains of healthy plants and remain dormant until crop is sown in the next season. Frequent rain showers and high humidity at flowering favour infection.

Fig. 16.7 Loose smut disease of wheat

16.3.4.2 Pathogen

The pathogen responsible for loose smut of wheat is *Ustilago segetum* var. *tritici*, which was earlier known as *Ustilago tritici* (Pers.) Rostr. Spores which are produced in abundance are pale olive, spherical to oval, 5–9 μm in diameter and have minute echinulate walls. On germination they do not produce sporidia but the promycelium gives rise to threads.

16.3.4.3 Disease Cycle

Spores fall on the stigma and germinates and produce promycelium from which infection threads enter the style and grow intercellularly towards the ovary. By the tenth day successful entry is made to the ovule, where the germ tube branches and mycelium gets well ramified in it and in the embryo. Thus, the fungus resides in the kernel as the dormant mycelium. When the infected grains are sown the hyphae keeps pace with the apical meristem. At the time of ear formation, the fungus enters the reproductive phase. A high humidity of 65–85% and temperature of 23 °C is essential for maximum disease development.

16.3.4.4 Management

Efforts have been made to combat this disease using bio-control agents. It is thought that antagonistic microorganisms applied to seeds prior to planting produce antifungal metabolites and colonize the rhizosphere of seedlings, thereby reducing disease intensity (Dandurand and Knudsen 1993). The management approaches can be successful only if antagonists are compatible with pesticides or other control practices. *Trichoderma viride* (TV-5) strain which showed biocontrol potential against Loose smut pathogen, was found incompatible with Carboxin at 200 ppm concentration (Mondal et al. 1995). In a multilocation trial culture filtrate of *Trichoderma viride* along with half recommended dose of Carboxin was used which successfully controlled loose smut infection (Anon 1996).

The disease can be managed effectively by treating the seeds with systematic fungicides like carboxin (Vitavax), carbendazim (Bavistin 50 WP) at 2.5 g/Kg of seed and tebuconazole (Raxil 2 DS) at1.5 g/Kg of seed. The use of disease-free seeds for sowing is also important. The loose smut resistant bread wheat varieties like HS 277, VL 829, PBW 34, Halna and durum (d) wheat varieties like PDW 233, WH 896, HI 8498 and RAJ 1555 should be grown.

16.4 Maize Diseases

Maize (*Zea mays* L) is an important food and fodder crop. In India, maize is the third most important food crop after rice and wheat. It provides nutrients for humans and animals and serves as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and, more recently, fuel. India is the 4th in area and 7th largest producer of maize in the world contributing 3% of the global production. Among the major maize-producing states, Andhra Pradesh tops the list with the contribution of 17% to the total Indian maize production followed

by Rajasthan, Bihar and Maharashtra (Anon 2011). Various biotic and abiotic stresses, diseases and pests are playing a direct role in affecting production of maize and earnings. According to Payak and Sharma (1985), 61 diseases of both tropical and temperate origin in maize are important causing average yield loss of 13.2%, of which, foliar diseases alone cause 5% loss.

Maize suffers from 28 diseases in seedling stage (Bari and Alam 2004) in which 11 are seed-borne in nature (Debnath et al. 2012). Somda et al. (2008) recorded 10 pathogenic fungi on maize seeds and evaluated the rates of seed-to-seedling transmission of some fungal pathogens. The problem of seed rot and seedling blight is more common in temperate areas particularly due to low soil temperatures prevailing during sowing time. However, in the major tropical environment of India, rapid emergence of seedlings helps in avoiding seedling blight.

In general, reduction in plant stands, wilting, chlorosis, yellowing, slow growth, stunting in a random or circular pattern are the resultant of this group of diseases. More specifically seeds decay before emergence of seedlings in the cold soil (low temperature soil). But in warmer soil, post-emergence die-back as well as stunting of newly emerged seedlings are seen. Other specific symptoms include reddish or yellow discoloration of leaves, complete or partially rotten roots with firm or soft, brown-reddish to grey lesions or decay, discolored and soft coleoptiles, drying of leaf tips and sunken, discolored lesions on mesocotyl.

A variety of pathogens are associated with seed rots and seedling blights including *Pythium, Fusarium, Rhizoctonia, Acremonium, Colletotrichum, Penicillium, Sclerotium,* etc. Two bacteria *Erwinia* (= *Pantoea*) and *Pseudomonas* sp. are also known to be involved location wise. Nematodes can also damage corn seedlings especially in sandy soils. The seed-borne fungi recorded by Somda et al. (2008) were consisted of *Acremonium strictum* (infection ranging from 2% to 96%), *Bipolaris maydis* (1–30%), *Botryodiplodia theobromae* (1–17%), *Colletotrichum graminicola* (2–8%), *Curvularia* sp. (1–39%), *Exserohilum rostratum* (1–13%), *Fusarium moniliforme* (38–99%), *F. equiseti* (1–15%), *F. pallidoroseum* (1–23%) and *Phoma* sp. (2–50%). Few fungi namely *Aspergillus flavus* (1–99%), *A. niger* (1–99%), *Cladosporium* sp. (1–93%), *Penicillium* sp. (12–100%) and *Rhizopus* sp. (1–51%) were detected as the saprophytic fungi. The pathogens overwinter on maize crop debris or soil and are also carried on seeds. Usually these diseases are prevalent in densely sown fields, poorly drained, cold and wet soils. Planting of seeds too deep in wet soil, use of poor quality seeds enhances the seed rot and seedling blight.

Selection of proper sites and dates for planting helps in taking the advantage of warm soils. Destruction of old maize stalks and crop rotation helps in minimizing or avoidance of the diseases. Seed treatment with fungicides like Thiram and Captan at 2 g/Kg of seed improves seedling vigour and plant stand and increases yield by 8–10% (Laxminarayana et al. 1967). Among the new generation fungicides, effective seed treating fungicides are Metalaxyl-M (Apron) and Tebuconazole (Folicur). Effort towards the development of resistant varieties has not yet been made because fungicide treatment is a simple and cheap method of protection against this group of diseases. Out of them, Maydis leaf blight (MLB) and *Curvularia* leaf spot (CLS) are discussed here.

16.4.1 Maydis Leaf Blight

The disease is also known as southern corn leaf blight (SCLB) of maize. It was first reported by Drechsler (1925) on maize in Florida, and later it was reported on teosinte in Philippines. In India, the disease was first reported by Munjal and Kapoor (1960) from the specimens collected from Malda (West Bengal) by Butler in 1905. MLB disease is distributed all over the world in warm temperate to tropical (20–30 °C) maize-producing areas. In India, MLB disease is prevalent in Assam, AP, Bihar, Delhi, etc. The causal pathogen *Bipolaris maydis* had significantly negative impacts on cob weight. The loss in yield as per the report of Payak and Sharma (1978) was 0.3% in the susceptible cultivars by the race 'O' of *B. maydis.* Similarly, Sharma and Rai (2000) reported that maydis leaf blight caused by *B. maydis* qualifies as a major disease of maize capable of inflicting significant losses in productivity to an extent of 41%.

16.4.1.1 Symptom

MLB forms numerous lesions with the size of up to 2.5 cm long which are present mostly on the leaves (Fig. 16.8). Initially they are elliptical, later elongate further becoming rectangular shaped by restriction of the veins. The colour of the spot is cinnamon-buff (sometimes with a purplish tint) with a reddish brown margin and occasionally zonate coalescing and becoming greyish with conidia.

16.4.1.2 Pathogen

The disease is caused by *B. maydis.* (Nisikado and Miyake) Shoemaker and its teleomorph is *Cochliobolous heterostrophus* (Drechsl.) Drechsl. Initially this pathogen was named as *Helminthosporium maydis.* On the basis of perfect stage, this fungus was renamed as *Ophiobolus heterostrophus* which was subsequently transferred to *C. heterostrophus* (Drechsl.) (Drechsler 1934). *C. heterostrophus* (Drechsl) is a heterophillic fungus with two mating type alleles and are cross fertile. So far, three races of *B. maydis* viz., Race O, Race T and Race C have been reported. Race O and T differ in symptoms produced, cytoplasmic specificity, production of toxins, optimum growth temperature, reproductive rate and plant parts attacked (Hooker

Fig. 16.8 Maydis leaf blight disease

1972). Lesions produced by the 'T' strain are oval and larger than those produced by the 'O' strain. A major difference is that the 'T' strain affects husks and leaf sheaths, while the lesions of 'O' strain are restricted on the leaves. Three types of resistance mechanisms to *B. maydis* are known in maize, those are operating by qualitative and quantitative nuclear genes and the third type is influenced by cytoplasm. Race C is confined to China only and has a specific virulence on cms-C cytoplasm. Larger lesions are produced on leaves of plants with cms-T, cms-S or N-cytoplasm in both seedlings and adult plants. In India, Race O and T occur and the Race O is most prevalent. Moreover, Race T is unable to create any threat in India due to avoidance of male sterile lines in Indian maize programme.

16.4.1.3 Disease Cycle

The disease appears in hot and humid conditions during *Kharif* (June to September) maize. An environment with warm temperatures (30–35 °C) and a high humidity (›90%) level is particularly conducive to SCLB. The fungus *B. maydis* survives as mycelium and spores in maize debris left in the field. Wind helps in the dissemination of the pathogen.

16.4.1.4 Management

Use of resistant varieties is advocated as one of the cheapest means of disease management. Dhanju and Dass (2005) identified multiple disease resistant inbred lines, HKI-295, HKI-1354, HKI-1348-6 and HKI-488 which is used as parents for released/identified hybrids like HHM-1, HM-5 and HM-6, which are resistant to both maydis leaf blight and rust disease. Among the seventeen elite inbred lines screened, SC-24-(92)-3-2-1-1, Suwan 1 (S) C#-f-f and SC 7-2-1-6-1 were highly resistant and TCA24-13-f-1, SC 7-2-1-2-7 and CM 116 showed moderately resistant reaction (Sharma and Rai 2005). Out of 30 genotypes evaluated against MLB, none was immune; however African tall shows resistance and J-1006 exhibited moderate reactions and the rest of the genotypes were susceptible to highly susceptible (Kumar and Saxena 2008).

Infected crop debris should be ploughed down to reduce the disease inoculums. Nitrogen alone or in combination with phosphorus or potassium, or with both phosphorus and potassium reduces the disease incidence (Pal and Kaiser 2001). Among various plant extract studied in vitro, garlic clove extract was found highly effective in inhibiting the growth of *H. maydis* followed by Neem leaf and Tulsi leaf extract (Kumar et al. 2009). Pal and Kaiser (2003) found Chlorothalonil (60 mg/ml) and mancozeb + thiophanate-methyl (100 mg/ml) completely inhibited the growth and conidial germination of *Drechslera maydis*. In field experiment, application of Captan resulted in the lowest disease index. Foliar spray of Mancozeb at 2.5 g/l or Chlorothalonil at 2 g/l twice at 14 days interval is highly effective to restrict the disease spread on the foliar parts.

16.4.2 *Curvularia* Leaf Spot

Curvularia leaf spot (CLS) disease occurs sporadically with a minor importance, but it is becoming increasingly important. This foliar disease was reported from different parts of the world by considering association of various genera of the pathogen prior to erection of the genus *Curvularia* by Boedijn (1933). For example, *Helminthosporium curvulum* Saccardo was reported from Philippines (Mason 1928), *Acrothecium lunatum* (presently *Curvularia lunata* (Wakker) Boedijn) from Gold Coast (Togo) (Bunting 1926), *C. inequalis* (Sher) Boedijn (Mc-Keen 1952), *C. maculans* (Bancroft) Boedijn from North Carolina and Georgia (Nelson 1956) and from Brazil (Franco 1960) and *C. pallescens* Boedijn from Nigeria (Mabadeje 1969). It was first reported from India by Jain in 1962 from the leaves and grains of maize and paddy. Later in 1969, Mandokhoi and Basu Chaudhary (1972) found another leaf spot disease incited by *C. clavata* Jain in the maize cultivar 'Composite Jawahar' in Banaras Hindu University, Varanasi that considerably reduced the fodder value due to severe spotting on the leaves.

16.4.2.1 Symptoms

Numerous small, circular, necrotic or chlorotic spots surrounded by light coloured halo are developed on the leaves. Initially symptoms start as pin-head size translucent spots on the leaf lamina. Fully developed spots measure about 0.5 cm in diameter. The spots are sometimes coalescing and form larger necrotic patches on the leaves.

16.4.2.2 Pathogen

The disease is caused by *C. lunata*, *C. pallescens* and *C. maculans*. Several species of *Curvularia* were reported to be involved with these leaf spots. Conidiophores are simple, erect, brown, septate and apically geniculate. Conidia are developed singly at the tip of the conidiophores, four celled, clavate, pale brown and slightly curved. Their central cells are darker than the basal and apical cells. The sub-apical cell is larger than other cells. The apical cell is short and rounded. The fungus *C. lunata* produces a non-host specific furanoid toxin to cause disease in maize (Liu et al. 2009).

16.4.2.3 Disease Cycle

The disease appears in hot and humid conditions. Age of the host, rainfall, relative humidity, temperature etc. play important role in the development of CLS disease (Mandokhoi and Basu Chaudhary 1980b). According to them, the conidia of *C. clavata* developed in primary infection germinate fast by quickly utilizing rain moisture and cause fresh infection. The pathogen can live saprophytic life in organic debris present in soil producing sclerotia towards the end of the growth period that helps it to survive until the favourable season returns for disease development (Mandokhoi and Basu Chaudhary 1980a).

16.4.2.4 Management

In order to manage CLS disease, Saxena (2010) prescribed the following practices, namely, (1) the use of resistant cultivars such as Deccan-103, Deccan-101, Gaurav, Sartaj and Ganga-11, (2) 2–3 years crop rotation, (3) removal and burning of diseased crop debris and (4) two to three foliar sprays of Mancozeb at 2.5 Kg/ha. at 10 days' interval. According to Faiemisin and Okuyemi (1976), fungicides copper oxychloride, copper oxychloride + zineb and benomyl were more effective than mancozeb, zineb or maneb in restricting *C. pallescens* in vitro. However, only copper oxychloride + zineb and copper oxychloride alone effectively controlled *Curvularia* leaf spot on maize. Lal and Tripathi (1977) evaluated 13 fungicides both in vitro and in vivo against leaf spot caused by *C. pallescens* and found Difolatan at 0.15% as the best in controlling the disease and giving significantly higher grain yield.

16.5 Conclusion

Many pathogens are carried by and move together with the seed. Limited information is available on crop damage and yield losses caused by pathogens carried by seed. Further, less information is available about disease establishment in the field and the effect of seed-borne pathogens on crop production. Study on epidemiology and estimation of yield loss to estimate the risk could be strengthening. The threshold inoculum carried by a seed lot should be defined in terms of its effect on transmission and disease establishment. In recent years, there has been development in biological control of plant diseases and several products are also suitable for seed treatment. However, the products are not fully developed and they require further testing for efficacy and their practical application. Other alternative control methods are available including hot water, hot air, irradiation of seed for diseases like bunt where there is surface infection. These alternative methods are not used in practice but must be considered in future together with other control methods and possible integration with a differentiated use of chemical seed treatment. Seed-borne inoculum of many diseases is inconspicuous and cannot be detected by direct inspection of dry seed or even after examination of low-power binocular microscope. The strong and vibrant programme of detection and management of seed-borne disease with synergistic efforts of traditional and biotechnological means may lead to better management of these diseases.

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Seed-Borne Diseases of Important Oilseed Crops: Symptomatology, Aetiology and Economic Importance

17

P. P. Thirumalaisamy, K. S. Jadon, and P. Sharma

Abstract

Annual oilseed crops in India occupy an area of 27 million ha with the production of about 30 million tonnes. More than 70% of the oilseed cultivation occurs in resource-poor rainfed areas and is prone to several seed-borne diseases, which lead to low productivity. The reduction in seed germination due to seed rot and seedling mortality reduces the plant population level to below optimum; besides seed-borne pathogens contaminate the areas which were disease-free previously. Seed-borne diseases due to presence of pathogen either internally or externally on seed or on vegetative propagating materials or as concomitant contamination are potential threat to cultivation of annual edible oilseed crops. This chapter deals about symptomatology, aetiology and economic importance of seed-borne diseases of cultivated annual edible oilseeds, viz. peanut (groundnut), sesame, soybean, rapeseed-mustard, sunflower and safflower.

17.1 Introduction

Annual edible field-grown oilseed crops have wide adaptability and are grown under varied agroclimatic conditions. They occupy a special place in agricultural economies all over the world. Almost all such crops have a great potential for the diversification of major cropping systems in developing countries. Oilseeds occupy

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an important position in the agricultural economy of India. The country is the largest producer of oilseeds in the world and contributes 6–7% of the global vegetable oil production with 12–15% share in the area and 9–10% of the total edible oil consumption. In terms of acreage, production and economic value, oilseeds are second only to food grains. Besides the nine major oilseeds cultivated in India, a number of minor oilseeds of horticultural and forest origin, including coconut and oil-palm, are also grown. In addition, substantial quantities of vegetable oils are obtained from rice bran and cotton seed along with a small quantity from tobacco seed and corn (Jha et al. 2012).

In recent years, annual oilseed crops in India occupy in an area of 27 million ha with the production of about 30 million tonnes, whereas the total edible oil production in the country stood at 6–7 Mt (FAOSTAT 2013). Oilseeds area and output are concentrated in the states of Madhya Pradesh, Gujarat, Rajasthan, Andhra Pradesh and Karnataka. Among different oilseeds, groundnut, rapeseed-mustard and soybean account for about 80% of area and 87% of production of oilseeds in the country (Jha et al. 2011).

The growth in the domestic production of oilseeds has not been able to keep pace with the growth in the demand in the country. Low and unstable yields of most oilseed crops and uncertainty in returns to investment, which result from the continuing cultivation of oilseeds in rainfed, high-risk production environments, are the factors leading to this situation of wide demand-supply gap.

The major factors which hamper the improvement in productivity of oilseed crops in India are (i) cultivation of old and inferior varieties on by and large marginal lands under low-input and rain-dependent situations, (ii) flawed application of fertilizers, (iii) inefficient use of water resources and (iv) the biotic and abiotic stresses (Misra and Rathnakumar 2011).

Pest and diseases are major challenges to the cultivation of oilseed crops. Some of the seed-borne pathogens transmit the diseases systemically. The reduction in seed germination and increase in seed rot and seedling mortality reduces the plant population level to below optimum. Another adverse effect of seed-borne pathogens is that it contaminates the areas which were disease-free previously. So, these necessitate to concern about the seed-borne diseases, its economic importance and symptomatology for effective management practices.

17.2 Seed-Borne Diseases of Groundnut

Groundnut (*Arachis hypogaea* L.) is the third most important oilseed crop after rapeseed-mustard and soybean. India is the largest producer of groundnut in the world; however, average yields are low at 1360 Kg/ha (Misra and Rathnakumar 2011). Groundnut crop is prone to attack by numerous diseases to a much larger extent than many other crops. More than 55 pathogens including viruses have been reported to affect groundnut. Some diseases are widely distributed and cause economic crop losses. As the economic part is produced under the ground, soil-borne diseases are important due to infection of pathogen on pods and kernels and carry the inoculum

to the next crop. Of seed and soil-borne diseases, collar rot, stem rot, dry root rot and aflaroot have been realized to be the major limitations. The pre- and post-harvest losses due to aflatoxin contamination are a major challenge to the export. These diseases cause severe seedling mortality resulting in 'patchy' crop stand in sandy loam soils and reduce the yields from 25% to 40%. Besides, *Fusarium*, *Rhizopus* and *Pythium* are spread by infected seed and nevertheless causes minor damage to the crop. Among several virus diseases of groundnut reported from India, *Peanut mottle virus* has been reported to be seed-borne and seed transmitted.

17.2.1 Crown Rot/Collar Rot

17.2.1.1 Aetiology

Aspergillus niger Van Tieghem and *A. pulverulentus* (McAlpine) Thom cause diseases at all stages of the groundnut crop. The pathogen survives in the soil, in the decayed plant debris and seed. It may be carried on externally as seed-borne but mainly soil-borne inoculum serves as the primary source of infection. The fungus develops best at a temperature between 31 °C and 35 °C. During heavy and incessant rains, very high incidence of pre- and post-emergence seedling blight is observed. High temperature and humidity conditions prevailing in the first fortnight of July immediately after the rainfall enhance the seedling mortality. Generally, the disease appears within a month after sowing, occasionally in later stage plants also get dried. Late sowing, insect feeding, high soil temperature and drought stress in the first few weeks after sowing have been associated with the incidence of this disease.

17.2.1.2 Economic Importance

In India, collar rot was reported by Jain and Nenra (1952). It is prevalent in almost all groundnut-growing states principally in Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujarat, Maharashtra, Rajasthan, Karnataka, Punjab and Odisha, especially in sandy loam and medium soils. This disease is severe in *Kharif* than in *Rabi* (Ghewande 1983). In India, under Punjab conditions, the losses may amount to 40% to 50% in terms of mortality of plants (Aulakh and Sandhu 1970; Chohan 1972). The loss due to this disease was reported from 28% to 47% (Bakhetia 1983).

17.2.1.3 Symptomatology

The pathogen attacks the groundnut seeds before germination, causes pre-emergence rotting of seeds (seed rot) and also causes rotting of germinated seeds. After germination of seed and emergence from soil, the most common cause of loss is due to rotting at the hypocotyl tissues and rapid wilting of the seedlings (seedling rot/collar rot) (Fig. 17.1). The site of infection initially becomes water soaked, later turns into light brown due to rotting and bears abundant black conidia on the conidiophores. Mature plants may also be attacked on the stem below the soil and spread upwards along the branches causing wilting of either branches or whole plants (crown rot). Lesions developed on the dead dried branches are easily detached from the disintegrated collar region.

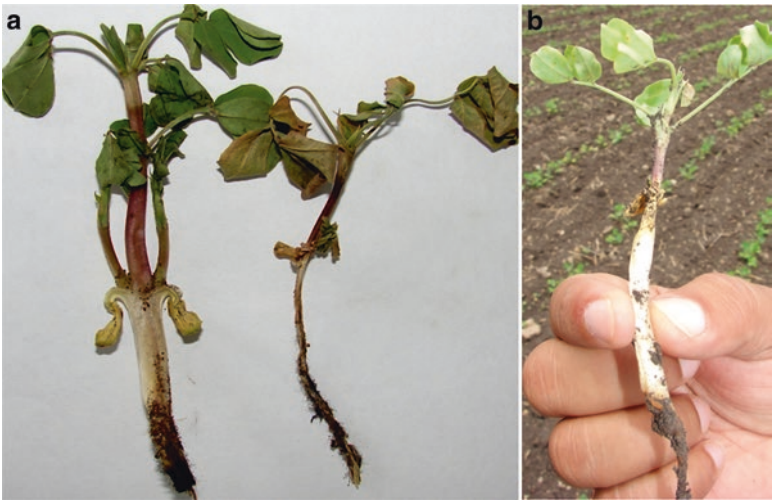

Fig. 17.1 (**a**) Crown/collar rot and (**b**) rotting of below hypocotyls region

17.2.2 Stem Rot

17.2.2.1 Aetiology

The disease is caused by *Sclerotium rolfsii* Sacc., a polyphagous plant pathogen, which attacks more than 500 plant species. The basidial stage is called *Athelia rolfsii*. The disease is initiated and spread through sclerotia present in the soil, dormant mycelia present in the infected seeds and also through the plant debris. Sclerotia produced by the pathogen can survive in soil for 4 years or more (soil inhabitant). The disease is also called as *Sclerotium* blight, *Sclerotium* wilt, Southern blight, Southern stem blight, Southern stem rot, white mould and *Sclerotium* stem rot.

17.2.2.2 Economic Importance

In India, stem rot occurs in all groundnut growing states, particularly severe in Gujarat, Maharashtra, Madhya Pradesh, Karnataka, Andhra Pradesh, Odisha and Tamil Nadu where it is estimated that over 50,000 ha of groundnut fields are infected with S. *rolfsii* (Mehan et al. 1995). In India, about 27% or more losses in yield of groundnut have been reported (Singh and Mathur 1953; Chohan 1974). Zaved et al. (1983) reported that *S. rolfsii* also causes indirect losses such as reduction in dry weight and oil content of groundnut kernels. This disease is most severe, particularly where irrigated groundnut cultivation is expanding in Marathwada region of Maharashtra and also in Saurashtra region of Gujarat where mostly mono-cropping and set furrow systems are practised. The disease incidence on farmers' field ranges from 0% to 60% particularly in Spanish bunch varieties in light soil and yield losses of over 25% have been reported (Mayee and Dattar 1988). Pod rots incited by *S. rolfsii* are also economically important in central and southern Maharashtra and Raichur area of Karnataka. Of late, this disease is assuming serious proportion in south Saurashtra zone of Gujarat particularly in Junagadh district both in medium

black and light calcareous soils. The survey conducted during *Kharif* 1996–1999 in farmers' fields of Junagadh district revealed that the average incidence of stem rot ranged from 14.3% to 24.0%. The post-harvest observations of 4 years (1996–1999) indicated that the average pod infection, seed infection and seed colonization was 23.4%, 9.5% and 3.6%, respectively.

17.2.2.3 Symptomatology

The disease may appear at any stage of the crop growth. Sudden wilting of whole plants or branches is the common symptom observed in 40–70 days crop. The diseased plants show dropping of leaves, withering and later turn to brown. At the site of infection, light brown rotten tissues entangled with white mycelia are visible. Mycelia bear abundant sclerotia which are initially pale yellow and later turn into brown to dark brown. Rotting pods along with white entangled mycelia on the surface of the pods are common symptoms observed at the time of harvest. Besides, the pathogen can attack on seeds (seed rot) and seedlings (seedling rot) (Fig. 17.2).

17.2.3 Dry Root Rot/Dry Wilt

17.2.3.1 Aetiology

Dry root rot is caused by *Macrophomina phaseolina* (Tassi) Goid, the sclerotial stage of which is known as *Rhizoctonia bataticola* (Taub.) Butl. Dry root rot has wide host range. The pathogen is facultative saprophyte and a soil dweller. Infected soil, plant debris and pods serve as the sources of inoculum. The sclerotia are disseminated through plant debris, soil, infected pods, shell and kernel.

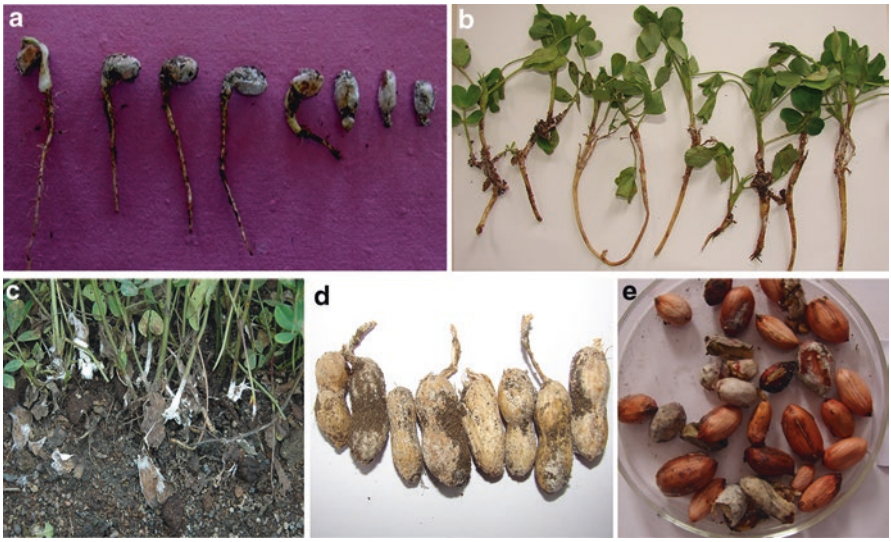

Fig. 17.2 Various symptoms of stem rot of different plant parts and at different stages: (**a**) rotting of germinating seedlings and seeds, (**b**) seedlings, (**c**) stem rot, (**d**) pod rot and (**e**) kernel rot

17.2.3.2 Economic Importance

Dry root rot or charcoal rot is sporadic in nature and occurs in Gujarat, Rajasthan, Uttar Pradesh, Tamil Nadu, Andhra Pradesh and Maharashtra. Recently, the dry root rot incidence is severe in red soil areas of Andhra Pradesh, Karnataka and Tamil Nadu. The pathogen causes severe seedling mortality resulting in patchy crop stand, thus reduces optimum plant population. Root rot is higher in bunch varieties as compared to the spreading type.

17.2.3.3 Symptomatology

The disease may appear at any stage of the crop growth. But generally, it appears in 50–60 days old crop when there is stress condition. Water soaked necrotic spots appear on the stem just above the ground level. The lesions darken as the infection spreads upward to the aerial parts and down into the roots. If the initial lesion girdles the stem, wilting follows. The entire stem becomes shredded, and with the development of sclerotia, it becomes black and sooty in appearance. Roots, pegs and pods also rot and become covered with sclerotia. Roots are commonly attacked in association with stem rots and wilt. Occasionally the roots alone are attacked. In this case, the tap root turns black and later becomes rotten and shredded (Fig. 17.3). The kernels turn black with abundant sclerotia internally and externally on the testa and shell. The optimum temperature for seedling infection is 29 °C–35 °C, for pods invasion is between 26 °C and 32 °C. The symptoms of the leaf infection are characterized by marginal zonate and irregular spots. Minute spots are also quite common and expand into bigger wavy spots.

17.2.4 Yellow Mould or Aflaroot

17.2.4.1 Aetiology

Aspergillus flavus (Link) Fries is commonly found in the seed of both rotten and apparently healthy pods of groundnut. This disease is seed- and soil-borne. It is usually associated with drought stress or high moisture conditions. The pathogen can tolerate low soil moisture, and the fungus develops best at temperature between 25 °C and 35 °C.

Fig. 17.3 Dry root rot with pod rot

Fig. 17.4 (**a**) Aflaroot plant with healthy plant and (**b**) *A. flavus* on seeds and pod

17.2.4.2 Economic Importance

This disease is prevalent in almost all groundnut-growing states. It is very common during *Kharif*. Though the plant mortality is very less, infected plants are seen in most of the fields. However, compared to collar rot the disease severity is very low. A loss of 10%–20% in the emergence of seedlings due to seed-borne infection by *A. flavus* was reported in India by Aujla (1971). In fields, from 5% to 10% of the seedlings showed aflaroot symptoms (Chohan et al. 1970; Aujla 1971; Chohan 1974).

17.2.4.3 Symptomatology

Seed and un-emerged seedlings are attacked by the fungus. Decay is most rapid when infected seeds are planted. In some cases, the emerging radicle and hypocotyl are infected which decay rapidly. At seedling stage, cotyledons infected with the fungus show necrotic lesions with reddish brown margins. This necrosis terminates at or near the cotyledonary axis. Under field conditions, the diseased plants are stunted and are often chlorotic (Fig. 17.4a). The leaflets are reduced in size with pointed tips, widely varied in shape and sometimes with vein clearing. Such seedlings lack a secondary root system, a condition known as 'aflaroot'. *A. flavus* is recognized by its yellowish green colour, and its colonies develop on over mature and damaged seeds and pods (Fig. 17.4b).

17.2.5 Peanut Clump Disease

17.2.5.1 Aetiology

The peanut clump disease of groundnut in India is caused by the *Indian peanut clump virus* (IPCV) belonging to the genus *Pecluvirus*, family *Virgaviridae*. IPCV consists predominantly of two single-stranded RNA species with RNA1 and RNA2 (Reddy et al. 1985), and both were needed for production of lesion. The virus is transmitted by seed as well as by the fungus. Seed transmission in the field-infected groundnut plants ranged from 3.5% to 17%, depending on the genotype. The transmission frequency of the virus through seed was 48%–55% (Reddy et al. 1998). IPCV was reported to be transmitted by the obligate fungal parasite (*Polymyxa graminis*) which is soil-borne (Ratna et al. 1991).

Fig. 17.5 (**a**) Distribution of peanut clump in field and (**b**) peanut clump disease

17.2.5.2 Economic Importance

The yield losses due to this disease may go up to 60% in late infected plants since such plants do not produce pods. The virus has a wide host range and many weeds in groundnut fields also get infected by the virus. Peanut clump disease has been reported from Punjab, Gujarat, Andhra Pradesh, Uttar Pradesh and Rajasthan on crops grown in sandy soils.

17.2.5.3 Symptomatology

The peanut clump disease appears in patches in the field and reappears in the same position in progressively enlarged patches, in succeeding years (Fig. 17.5a). The patchy appearance of the disease and its occurrence year after year in almost the same area of a field is due to the soil-borne nature of the vector, *P. graminis* and its survival as highly resistant resting spores. Typical symptoms are severe stunting of the plant apparent first on newly emerged leaves of 2- to 3-week-old seedlings (Fig. 17.5b). The newly emerged leaves show mottling and chlorotic rings. Later, the infected leaves turn dark green with faint mottling. Infected plants ultimately appear bushy and have small, dark green leaves and usually produce several flowers on erect petioles. The number and size of pods are reduced, resulting in small seeds. Root systems of infected plants get reduced in size and become dark.

17.2.6 Peanut Mottle

17.2.6.1 Aetiology

Peanut mottle virus (PeMoV) is a plant pathogenic virus of the family *Potyviridae*, genus *Potyvirus*. PeMoV is a flexuous, non-enveloped, filamentous virus with particles ranging from 723 nm to 763 nm in length and 12 nm in diameter (Pietersen and Garnett 1992). In infected plant cells, the virus makes characteristic *Potyvirus* cylindrical inclusions that are visible in the light microscope with proper staining. Infected groundnuts are considered to be the primary sources of PeMoV (Prasada Rao et al. 1993) and other nearby leguminous crops become infected from this crop. The virus is seed transmitted in a range from 0.1% to 3.5% depending on the type

of cultivar (Bashir et al. 2000). Adams and Kuhn (1977) reported that seed transmission is due to the presence of the virus in the embryo. PeMoV is transmitted in a stylet borne (non-persistent) manner by *Aphis craccivora*, *Myzus persicae*, *A. gossypii*, *Hyperomyzes lactucae*, *Raphalosiphum padi* and *R. maidis.* PeMoV occurs in several important legume crops, including groundnut, bambara groundnut and soybean and weeds. The virus has been isolated from *Pisum sativum*, *Glycine max* and forage legumes and a few weed hosts like *Cassia obtusifolia*, *C. leptocorpa*, *C. occidentalis* and *Desmodium canum* in nature. Nischwitz et al. (2007) reported about the virus infection on the rhizoma peanut (*Arachis glabrata*).

17.2.6.2 Economic Importance

In India, the virus was reported by Reddy et al. (1968) and subsequently this disease has been reported to occur on *Rabi*/summer groundnut from Andhra Pradesh, Gujarat and Maharashtra. Surveys conducted in *Rabi*/summer of 1983 revealed that the incidence of the disease ranged from 3.7% to 40% in cultivated bunch varieties (Ghewande 1983). Seed transmission appears to be the most important source of PeMoV for groundnut and most commercial peanut seed lots have a low frequency (< 15.0%) of seed infection. However, a frequency as low as 0.1% will provide about two infected seedlings per 100 m^2 in a field. Aphids are efficient vectors of PeMoV and transmit the virus rapidly to nearby plants (Kuhn and Demski 1984). The disease can cause up to 30% loss in yield (Kuhn and Demski 1975).

17.2.6.3 Symptomatology

Mild mottling and vein clearing in newly formed leaves, upward curling and interveinal depression with dark green islands in older leaves are characteristic diagnostic symptoms. Infected plants are not severely stunted and older plants seldom show typical disease symptoms. Some pods from plants infected with PeMoV may be smaller than normal and have irregular, green to brown patches with discolouration in the seeds.

The following diseases are minor and cause pod rot complex at the time of pod development and maturation in addition to seed and seedling rot.

17.2.7 *Fusarium* Wilt

17.2.7.1 Aetiology

Fusarium oxysporum Schl. emend Snyd. & Hans., *F. solani* (Mart), App. & Wr. emend Sny. & Hans.

17.2.7.2 Symptomatology

Germinating seeds are attacked by *Fusarium* spp. shortly before emergence. Rooting of germinated seeds covered with sporulating mycelium is common. Damping off symptoms characterized by brown to dark brown water soaked, sunken lesions on the hypocotyl appear which later encircle the stem and extend above the soil level. Roots

are also attacked, especially the apical portions. Infected site of root and epidermis are peeled off. The affected seedlings become yellow and wilted. The leaves turn greyish green and the plants dry. The roots and stem show internal vascular browning and discolouration. These fungi are also commonly associated with pod rot.

17.2.8 *Rhizoctonia* Diseases

17.2.8.1 Aetiology
Rhizoctonia solani Kuhn.

17.2.8.2 Symptomatology
Germinating seeds are invaded by the seed- or soil-borne inoculum, which may result in pre-emergence death. The fungus is commonly associated with pre- and post-emergence damping off and pod rots in India.

17.2.9 *Pythium* Diseases

17.2.9.1 Aetiology
Pythium myriotylum Drechsler, *P. debaryanum* Drec. Hesse, *P. butleri* Subramaniam.

17.2.9.2 Symptomatology
Pythium species cause a number of diseases of groundnut including seed rot, damping-off, root rot, vascular wilt and pod rot. Germinating seeds, before emerging from the soil, become rotten. A loose mat of white mycelium covers the surface of the rotting tissue. Severely infected seeds are reduced to a dark brown pulpy mass. At seedling stage, infected plants become stunted with pale green leaves which subsequently turn brown, dry up and are shed. The root system is greatly reduced due to rotting of lateral, fibrous and tap roots. In advanced stages, the cortical tissues turn brown and disintegrate leaving a non-functional vascular skeleton. Infection may occur at the time of pod maturation leading to pod rot.

17.2.10 *Rhizopus* Seed and Seedling Rot

17.2.10.1 Aetiology
Rhizopus arrhizus Fischer, *R. oryzae* Went & Gerlings, *R. stolonifer* (Ehrenberg ex. Fr.) Vuillemin.

17.2.10.2 Symptomatology
Rhizopus spp. may attack on seed and pre-emerged seedlings. The infected seed and seedlings are reduced to a dark brown or black spongy mass of rotted tissue covered with a mat of mycelium on which masses of grey-black spores are produced.

17.3 Seed-Borne Diseases of Sesame

Sesame (*Sesamum indicum* L.; Family: Pedaliaceae) is a flowering plant in the genus *Sesamum*. Numerous wild relatives occur in Africa and few in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds present in the capsule. Sesame seed is one of the oldest oilseed crops known in India, cultivated and domesticated during Harappa and Anatolian eras (Bedigian 2003).

Many factors are responsible for low productivity of the crop. Diseases, insect pest and abiotic stresses are major factors, among which the diseases are most important causing nearly 100% loss. The important fungal diseases includes *Phytophthora* blight (*Phytophthora parasitica* var. *sesami*), charcoal rot (*Macrophomina phaseolina*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *sesami*), *Alternaria* leaf spot (*Alternaria sesami* and *A. alternata*), white spot (*Cercospora sesami*), powdery mildew (*Oidium* spp., *Sphaerotheca* spp., *Leveillula taurica* and *Erysiphe cichoracearum*), brown angular leaf spot (*Cercoseptoria sesami*), aerial stem rot (*Helminthosporium sesami*), etc. The bacterial diseases include bacterial leaf spot (*Pseudomonas syringae*) and bacterial blight (*Xanthomonas campestris* pv *sesami*). The crop is affected by viral diseases, viz. leaf curl virus and mosaic virus, and by phytoplasma like organisms causing phyllody (Chattopadhyay et al. 2015). Among several diseases reported in sesame, the important seed-borne pathogens causing diseases in sesame crop are described below.

17.3.1 *Alternaria* Leaf Spot and Blight

17.3.1.1 Aetiology

The pathogen *Alternaria sesami* (Kawamura) Mohanty and Behera is externally as well as internally seed-borne. From infected capsules, *A. sesami* can penetrate into the seed coat and may survive up to 11 months, and it can also perpetuate in infected debris for nearly 11 months under field conditions (Agarwal et al. 2006; Naik et al. 2007). Secondary spread of the disease occurs through chain of conidia produced on conidiophores. Temperature of 20–30 °C and high humid conditions are favourable for infection and disease development. The disease becomes most severe on plants established from seeds with 8% infection, and the disease severity increases with increased seed infection level (Ojiambo et al. 2000, 2003, 2008). The toxin, tenuazonic acid produced by *A. sesami* (Rao and Vijayalakshmi 2000) is suspected to play a role in disease development.

17.3.1.2 Economic Importance

The disease was first reported from North Caucasus region of former Soviet Union in 1928. Later it was reported from Japan, India, the United States, Kenya, Uganda, Egypt, Nigeria and Pakistan. Occurrence of disease in epidemic level was reported in Florida (1958), Mississippi (1962) and states of Odisha (1957) and Maharashtra (1975) of India. The plants are killed due to severe defoliation and stem infection.

Fig. 17.6 (**a**) *Alternaria* spot on leaf and (**b**) stem, petiole and on capsule

Due to the disease, yield losses from 20% to 40% were reported in sesame crop grown in Utter Pradesh state of India, and about 0.1–5.7 g seeds per 100 g fruits were lost in Karnataka state of India.

17.3.1.3 Symptomatology

The pathogen attacks all parts of the plant at all stages. Brown to black, round to irregular and often zonate lesions measuring up to 1 cm diameter are produced on the leaves (Fig. 17.6). In severe attacks, several leaf spots may coalesce and the leaves dry out and fall off (N'Gabala and Zambettakis 1970). Stem lesions are either in the form of dark brown spots or streaks. Dark brown, circular lesions are produced on the capsules (Yu et al. 1982) which can cause the capsule to drop (N'Gabala and Zambettakis 1970). The most visible symptoms are the leaf spots which are dark, irregular patches mostly on the edges and tips of the leaves, but the stem rots can be more significant. Milder attacks cause only defoliation; in severe cases, the plant may die. Kushi and Khare (1979) reported that *A. sesami* can cause seed rot, pre- and post-emergence losses as well as stem rot and leaf spots. Histopathology of sesame stem showed that the fungus was present in epidermis, hypodermis and cortical parenchyma tissue as the symptoms became visible by naked eye 10 days after inoculation. As the disease progress, the fungus moved from cortical parenchyma to vascular bundle, xylem and phloem. Later on, it completely overlapped the vascular bundle and entered in the pith. When necrotic lesion appeared, fungus was present abundantly in epidermis, hypodermis, cortical parenchyma, vascular bundles and in pith. Due to its excessive growth and complete overlapping of cells, disorganization or destruction of cells of sesame took place. It was concluded that the *A. sesami* was not a tissue limited pathogen; instead of this it spread into all tissues of stem from epidermis to pith (Ajmal et al. 2016).

17.3.2 *Cercospora* Leaf Spot or White Leaf Spot

17.3.2.1 Aetiology

Cercospora leaf spot, caused by the fungus *Cercospora sesami* Zimmerman is seed-borne. The pathogen also perpetuates in the infected plant debris in soil. Secondary spread of the disease occurs through conidia produced on conidiophores. The conidia are spread to healthy plants through rain, irrigation water and wind. Germination occurs in humid conditions, usually during late spring and summer, and fungal growth is encouraged when leaves are frequently damp.

17.3.2.2 Economic Importance

The disease is reported from many sesame growing countries. Due to the disease, yield loss from 22% to 53% was reported in Nigeria (Enikuomehin et al. 2002), and 20% yield loss was reported in India (Mohanty 1958; Patil et al. 2001).

17.3.2.3 Symptomatology

The fungus *C. sesami*, infects all above ground parts of the plant, resulting in complete defoliation which leads to severe economic losses. The disease, which affects leaves as early as 4 weeks after planting, starts as small pinhead-sized cottony spots on the infected leaves surrounded by a blackish purple margin. These spots gradually spread on the lamina and can extend up to 4 mm in diameter. On petiole, the spots are elongated and on capsule, the spots are circular, brown to black in colour. Extensive infection leads to defoliation and damage of capsules before the plant reaches maturity which can result in yield losses of 20% to 50%.

17.3.3 Bacterial Leaf Spot

17.3.3.1 Aetiology

The pathogen *Pseudomonas syringae* pv. *sesami* is gram-negative aerobic rod-shaped bacterium with one or more polar flagella. The bacterium is internally seed-borne, invades all areal parts of the plant including pods, reaches up to inner seed coat of the kernel and survives there up to 11 months. The pathogen also survives in the infected plant debris and in soil for shorter period. The secondary spread of the disease occurs by rain water.

17.3.3.2 Economic Importance

The disease was first reported from Bulgaria in 1903 as black rot. The disease is reported from Brazil, China, Greece, India, Somali Republic, Japan, Korea, Pakistan, Mexico, South Africa, Sudan, Tanzania, Turkey, Uganda, the United States, Yugoslavia and Venezuela. Infected seed is the primary source for disease appearance. Under artificially infected condition, 21%–27% yield loss was reported in India. Capsule loss of 60% was reported in the infected plants.

17.3.3.3 Symptomatology

Water-soaked yellow specks are formed on the upper surface of the leaves. They enlarge and become angular as restricted by veins and veinlets. The colour of spot may be dark brown with shiny oozes of bacterial masses. Light brown angular spots with dark purple margin appeared in the interveinal area of leaves. Hayward and Waterson (1998) reported *P. syringae* pv. *sesami* produced blackish-brown spots, which extended along whole length of the stem. Veins delimit angular spots on leaves. Defoliation and death of plant may occur in severe leaf and stem infections under natural condition. Sunken and shiny spots may appear on the capsules. Early capsule infection renders them black and seedless. *P. syringae* pv. *sesami* is not xylem-limited pathogen and did not systemically transfer from inoculated petiole to leaf but infect some phloem tissues. It was concluded that leaf spot symptoms appeared due to infected mesophyl tissues and damaged chloroplast membrane due to the action of chlorosis producing toxins. Further, chlorosis producing toxin can contribute significantly to pathogen virulence, presumably by inhibiting photosynthesis.

17.3.4 Bacterial Blight/Angular Leaf Spot

17.3.4.1 Aetiology

The bacterium *Xanthomonas campestris* (Pammel) Dowson pv. *sesami* (Sabet and Dowson) Dye is a gram-negative rod with a monotrichous polar flagellum. The bacterium remains viable in the infected plant tissues; however, it lives relatively for shorter period (4–6 months). The bacteria is internally seed-borne, survive up to 16 months in seed which is the main source of inoculum for disease development and the secondary spread occurs through rain splash and storms. A weed plant, *Acanthospermum hispidum*, is reported to be susceptible and acts as a source of survival of the bacteria in its dried leaves from year to year.

17.3.4.2 Economic Importance

The disease was first reported from Sudan, later from India and Venezuela. The disease is destructive in nature and causes severe economic yield losses. Vijayat and Chakravarti (1977) reported 60% loss in the capsules due to blight under field conditions in Turkey, while through artificial inoculation in the field, the disease caused 21–27% loss of yield in India. About 20% loss in yield has been reported from Madhya Pradesh, India by Shukla et al. (1972).

17.3.4.3 Symptomatology

The disease appears in all stages of the crop. It attacks the cotyledons after germination and causes drying. In growing plants, water-soaked spots appear initially on the under surface of the leaf and then on the upper surface, often bordered by a small zone of lemon yellow tissue. Necrotic spot increase in size and, become angular and restricted by veins and dark brown in colour. Several spots coalesce together forming irregular brown patches and cause drying of leaves. The reddish-brown lesions may also occur on petioles and stem. In case of severe infection, dead leaves defoliate. It may also attack on capsule causing oval, slightly raised brown spots.

17.3.5 *Fusarium* Wilt

17.3.5.1 Aetiology

Fusarium oxysporum (Schelt.) f. *sesami* Jacz is restricted to cause disease in sesame alone. Another species, *F. proliferatum* causing sesame wilt disease was reported first time from Iran. The fungus is seed- as well as soil-borne, and the pathogen survives for longer period in soil. The seed transmission of the disease is in the range of 1–14% depending on the severity of systemically infected sesame plants. High soil temperature to a depth of 5–10 cm coupled with 17–27% water holding capacity during dry periods favours the development of the disease. Drought stress predisposes the sesame plant to the wilt disease.

17.3.5.2 Economic Importance

Fusarium wilt in sesame was first reported from North America in 1950. Since 1950, the disease is reported from Egypt, Former Soviet Union, Malawi, the United States, Columbia, Iran, India, Greece, Japan, Korea, Israel, Venezuela, Pakistan, Peru, Porto Rico and Turkey. Epidemic occurrence of *Fusarium* wilt in sesame was reported in 1959 from Venezuela and from the United States in 1961 and again in 1964.

17.3.5.3 Symptomatology

The pathogen attack at any stage of the sesame crop. In early stage, it causes damping-off disease. In later stage of crop, yellowing is the first visible symptom produced on leaves. Leaves become yellowish, droop and dry. Inward rolling of edges of leaves, drying of terminal tips, become shrunken and bent over are symptoms associated with this disease. In case of severe infection, the entire plants become dry and defoliated. Partial wilting, i.e. death of branches, alone is also reported in mature sesame plants. A blackish discolouration in the form of streak is conspicuous in the diseased plant. Discolouration of vascular system is also observed in the roots of infected plant. Fruiting bodies (sporodochia bearing macroconidia) are scattered on the entire dried diseased plant stem as well as on capsule.

17.3.6 Dry Root Rot or Charcoal Rot

17.3.6.1 Aetiology

The pathogen *Macrophomina phaseolina* (Tassi) Goid (Sclerotial stage: *Rhizoctonia bataticola*) produces dark brown, septate mycelium showing constrictions at the hyphal junctions. The sclerotia are minute, dark black and 110–130 µm in diameter. The pycnidia are dark brown with a prominent ostiole. The conidia are hyaline, elliptical and single celled. Day temperature of 30 °C and above and prolonged drought followed by copious irrigation are conducive for infection and disease development. The fungus remains dormant as sclerotia in soil as well as in infected plant debris in soil. The infected plant debris also carries pycnidia. The fungus primarily spreads through infected seeds which carry sclerotia and pycnidia. The fungus also spreads through soil-borne sclerotia. The secondary spread is through the conidia transmitted by wind and rain water.

17.3.6.2 Economic Importance

The disease was reported from many sesame growing countries. Seedling mortality due to seed-borne infection reduces optimum plant population in the field. About 5–100% loss in yield was reported due to the disease.

17.3.6.3 Symptomatology

The disease symptom starts as yellowing of lower leaves, followed by drooping and defoliation. The stem portion near the ground level shows dark brown lesions and bark at the collar region shows shredding. The sudden death of plants is seen in patches. In the grown-up plants, the stem portion near the soil level shows large number of black pycnidia. The stem portion can be easily pulled out leaving the rotten root portion in the soil. When the infection spreads to pods, they open prematurely and immature seeds become shrivelled and black in colour. Minute pycnidia are also seen on the infected capsules and seeds. The rotten roots as well as stem tissues contain a large number of minute black sclerotia. The sclerotia may also be present on the infected pods and seeds.

17.3.7 *Phytophthora* Blight

17.3.7.1 Aetiology

Phytophthora parasitica (Dastur) var. *sesami* Prasad causes *Phytophthora* blight in sesame alone. Mycelia and chalmydospores survive in soil and are the primary sources for disease initiation. Sporangiophores emerge in groups by rupturing epidermis or through stomata and bear zoospores which are responsible as secondary source of inoculum for infection and disease spread. In addition, infected seeds also play a crucial role in disease spread. However, there are reports that the fungus reduces the seed viability but it is not seed-borne. Heavy rains for 2 weeks and high humidity for 3 weeks favours the quick appearance and spread of the disease when soil temperature is 28–30 °C; however, above 37 °C, it fails to infect the crop. Heavy soils as well as application of moderate dose of nitrogenous fertilizer favours high incidence of the disease in sesame.

17.3.7.2 Economic Importance

The disease was first reported from India by E. J. Butler in 1918. Presence of disease has now been reported from Venezuela, Argentina, Egypt, Dominican Republic, Iran and Sri Lanka. The mortality of the plant due to the disease may be as high as 72–79% and losses in sesame yield ranges from 51% to 54% in Assam.

17.3.7.3 Symptomatology

Disease can occur at all stages of the plant and symptoms appear on all aerial parts of the diseased plant. Initial symptom is the appearance of water-soaked spots on leaves and stems. The spots are chestnut brown in the beginning but later turn to black. Premature leaf fall occurs. In humid weather, severity of disease increases by spread of brownish discolouration on leaves and stem; main root is also affected.

White mycelial growth of the fungus can be seen on the infected areas including on the capsule. Diseased plants are easily pulled out and they produce shrivelled seeds and give blighted appearance. The pathogen is reported to be inducing viviparity, i.e. emergence of the radicle, hypocotyls and cotyledons through the seed coat within the pod in 25.0–48.8% pods and 27.1–36.1% seeds.

17.3.8 *Corynespora* Blight

17.3.8.1 Aetiology

The pathogen *Corynespora cassiicola* (Berk. and Curt.) Wei survives through infected seed and plant debris. The conidia produced by the pathogen serve as secondary source of inoculum for disease spread within the crop season.

17.3.8.2 Economic Importance

The disease is reported in Tanzania, India, the United States, Columbia and Venezuela. The pathogen can also infect cowpea, lablab, soybean and tomato.

17.3.8.3 Symptomatology

Brown to dark brown irregular shaped spots appear on leaves and stem. The spots enlarge in later stages with dark brown margin and light centre, giving blotchy appearance. Extensive defoliation occurs, later plants die. Lesion increases in size on the stem in both ways up to 10–15 cm and bends irregularly. Canker of various sizes also appears on the stem with straw coloured warty growth on centre. Stem cracking has also been observed in the matured plants.

17.4 Seed-Borne Diseases of Soybean

Soybean (*Glycine max* (L.) Merrill) the ‘golden bean’ is one of the most important oilseed crops known for its excellent protein (42–45%), oil (22%) and starch content (21%). It is good source of vitamin B complex, thiamine and riboflavin. Soybean protein is rich in valuable amino acids like lysine (5%) in which, most of the cereals are deficient. Soybean can substitute for meat and to some extent for milk (Endres et al. 2013). In spite of phenomenal increase in area under soybean production, its productivity remains low because of lack of quality seeds. Low yield and productivity of soybean in India is also mainly due to various diseases and pests occurring in the field and causing yield losses. One of the major constraints in the endeavour of increasing productivity of soybean is its susceptibility to a large number of diseases caused by fungi, bacteria, viruses and nematodes. Seeds of soybean are known to harbour several species of seed-borne fungi, viz. *Cercospora kikuchii*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum*, *Colletotrichum dematium*, *Curvularia lunata*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Penicillium* sp. and *Rhizopus stolonifer* were found in germinating seeds and seedlings of soybean (Shovan et al. 2008). Disease-free quality seed

production in soybean is utmost important to sustain the productivity and to maintain the quality of the crop. The infected seeds fail to germinate or seedlings and plants developed in the field from infected seeds may escape the early infection but often may become infected at later stages of the crop growth. Besides, pathogens can spread over a long distance and uninfected field may also become infected by use of infected seeds. The frequency in occurrence of such potentially pathogenic fungi on soybean cultivars poses a potential threat in crop production programme. Transmission of the pathogen through seed is also known as a means of spread of disease into new areas and new countries. In India, although 40 fungal pathogens have been identified in soybean crop, only a few of them are economically important. Here, information on seed-borne pathogens causing seed-borne diseases in soybean is provided in brief.

17.4.1 Anthracnose

17.4.1.1 Aetiology

The primary pathogen that causes anthracnose is *Colletotrichum truncatum* (Schw.) Andrus and Moore, but *Glomerella glycines* and other fungi may also be associated with anthracnose. These pathogens overwinter in infected crop residue and infected seeds, and thus may be seed-borne. The fungus has a wide host range that includes alfalfa, ragweed and velvet-bean.

17.4.1.2 Economic Importance

It is reported to be a serious disease in Argentina, Austria, Brazil, China, India and Zambia. The disease typically has minimal effect on yield, but it can reduce yields, plant stand and seed quality. Although the symptoms of anthracnose can be mistaken for pod and stem blight, the symptoms are different and both diseases may be present on the same plant late in the season. The combined attack of the disease with frogeye leaf spot results in a yield loss of soybean yield in the range of 23.7%–32.5% in India (Mittal 2001). In addition to yield reduction, *C. truncatum* may affect seed quality, reduction in oil content in the range of 18%–27%, reduced seed germination up to 29.2% and viability by 26.9% coupled with lower seedling vigour (Galli et al. 2005, Nema et al. 2012).

17.4.1.3 Symptomatology

Infected seeds may not germinate. Dark, sunken cankers can occur on the cotyledons, epicotyl and radicle of seedlings to cause pre and post-emergence damping-off. Anthracnose is a stem disease that occurs during wet, warm and humid conditions, although symptoms are often not seen until plants reach maturity. The most common symptoms are seen late in the season as the plants approach maturity. Irregular brown spots develop in a random pattern on stems and pods. The infected areas are covered with tiny black spines (setae) that can be seen with a 10X hand lens. Brown cankers can appear on petioles and cause defoliation. Infection of pods

results in few or small seeds per pod. Infected leaves may develop brown veins and curl up. In older plants, the stems, pods and leaves may be infected without showing symptoms until the weather is warm and moist or plants reach maturity. Infected seeds may have no symptoms or may develop brown or grey areas with black specks. Seeds infected by anthracnose may not germinate. Infected seedlings develop dark, sunken cankers on the cotyledons, epicotyl and radicle that cause damping-off (Begum et al. 2007, 2008, 2010; Galli et al. 2007).

17.4.2 Bacterial Blight

17.4.2.1 Aetiology

The bacteria *Pseudomonas syringae* pv. g*lycinea* (Coerper) Youn et al. also infects snap bean and lima bean in addition to soybean. The primary foci in crops derive from seed-borne infection and the pathogen overwinters in crop residue can become secondary source for disease spread. Cool, wet weather and rain storms favour disease development. Disease progress stops in dry and hot conditions. The disease spreads by wind and rain in the fields as well as by intercultural practices when foliage is wet.

17.4.2.2 Economic Importance

This disease usually occurs at low levels that do not result in yield loss.

17.4.2.3 Symptomatology

Bacterial blight on soybean is a widespread disease, common during cool and wet weather. Bacterial blight can occur on all above ground plant parts, but is most evident on leaves in the mid to upper canopy. The primary foci in crops derive from seed-borne infection that inhibit germination and on cotyledons cause marginal lesions that enlarge and become dark-brown and necrotic. Often, the lesions are covered, particularly on their underside, with a film of a pale greyish bacterial slime that can dry to a thin silvery crust. The pathogen overwinters in crop residue and can become secondary source for disease spread.

Infections begin as small, angular, water-soaked spots that turn yellow and then brown as the tissue dies. The spots darken and are surrounded by yellowish-green halos visible on upper surface of leaves. Spots often merge to form large, dead patches on the leaves. The dead tissue may fall out giving the leaves a ragged appearance. These infected leaves usually remain on the plant. Infection can also occur on stems, petioles, pods and seeds in infected pods. Infected seedlings may be stunted or killed in severe cases. Bacterial blight can be mistaken for *Septoria* brown spot. The two diseases can be distinguished by the presence of a halo around bacterial blight lesions. Both diseases can occur together on the same plants, but bacterial blight is most common on young leaves whereas brown spot is usually seen on older, lower leaves in the plant.

17.4.3 Bacterial Pustule

17.4.3.1 Aetiology

Bacterial pustule is caused by the bacterium *Xanthomonas axonopodis* pv. *glycines* (Nakano) Dye. This pathogen overwinters in unburied crop residue and on seeds. The pathogen can be transmitted by seed, and can be spread by wind-blown rain, rain splash and by machinery during wet conditions. Bacterial pustule can also infect snap beans. The disease can occur at any time during the growing season and is favoured by warm and wet weather conditions.

17.4.3.2 Economic Importance

Bacterial pustule is far less economically important than bacterial blight. Yield losses of up to 40% have been reported in certain parts of the world (Prathuangwong and Amnuaykit 1987). The disease is widespread in many European countries causing seed losses of up to 28%. It has also become important in India, Korea, Serbia, southern United States and Thailand.

17.4.3.3 Symptomatology

Bacterial pustule is most common in areas prone to frequent warm and wet weather. The disease rarely causes defoliation and reduced yields. Early symptoms of bacterial pustule look similar to bacterial blight. Symptoms begin as small light green spots (not water-soaked) with raised centres on the upper and lower surfaces of leaves. Tiny tan elevated pustules often develop in the centre of lesions, especially on the underside of leaf. Adjacent spots may coalesce and cause premature defoliation (Narvel et al. 2001). The pustules can be mistaken for those of soybean rust; however, bacterial pustule does not have an opening in the pustules or masses of spores like those of soybean rust. The lesions can grow together into large irregular brown areas. The infected areas may tear away from the leaf. Small raised spots may also develop on pods.

17.4.4 *Cercospora* Leaf Blight and Purple Seed Stain

17.4.4.1 Aetiology

Cercospora leaf blight is caused by the fungus *Cercospora kikuchii* (Matsumoto & Tomoyasu) M. W. Gardner. The pathogen overwinters on seed or infested debris. Infected seeds serve as a primary source of inoculum. The same pathogen causes purple seed stain. Infection and disease development are favoured by high humidity and warm temperatures. Plants are susceptible from flowering to maturity.

17.4.4.2 Economic Importance

The disease is reported to occur in almost all soybean-growing regions in the world. A reduction in seed quality can occur due to staining rather than yield losses. When infected seeds are planted, the fungus grows from seed coats and infects seedlings. In most cases, 7–13% reduction in emergence can occur in the field.

17.4.4.3 Symptomatology

The first symptom of *Cercospora* leaf blight is the development of light purple spots and areas on the top surface of leaves exposed to light. The discoloured areas expand and darken in colour to a reddish-purple or bronze. The infected leaves appear leathery and 'sunburned'. Red-brown spots may develop on both leaf surfaces. The spots may coalesce to form large necrotic areas on leaves, which eventually result in defoliation of infected leaves in the upper canopy. Reddish-purple lesions may also occur on petioles, stems and pods. Symptoms usually become apparent during seed set. Infected seeds have pink to purple discolouration on the seed coats.

17.4.5 Bud Blight

17.4.5.1 Aetiology

Tobacco ringspot virus (TRSV). Seed transmission is the most important route for transmission, but thrips, grasshoppers and dagger nematodes may be involved in transmission as well. This virus has a wide host range including pea, common bean and red clover. Fields next to clovers, pastures and weedy areas are favouring conditions for the disease.

17.4.5.2 Symptomatology

Young plants can be stunted. The terminal bud of plants at various ages can bend over to form a characteristic hook, and buds may become brown and fall off. The pith may develop a brown discolouration, and leaflets may be smaller than normal and may deform by cupping. Pods may have brown patches and may develop poorly and abort. Maturity can be delayed and plants may remain green until frost.

17.4.6 Downy Mildew

17.4.6.1 Aetiology

Downy mildew is caused by the Oomycete *Peronospora manshurica*. Common snap bean is also a host. The fungus overwinters on infected leaves and seeds, and can be transmitted by seed. Downy mildew is a widespread disease that occurs during periods of high humidity and moderate temperatures. The disease is typically superficial and causes no yield loss, but can cause defoliation of plants and reduced yields under rare conditions. Downy mildew can be distinguished from other foliar soybean diseases by the tufts of tan-coloured fungal growth on the underside of infected leaves.

17.4.6.2 Symptomatology

Downy mildew can occur on plants of all ages, although the disease is most common after flowering begins. Young leaves are most susceptible and infected leaves are often seen on the top of plants. The initial symptoms of downy mildew are small, light green spots on upper leaf surfaces. The spots enlarge and turn pale to bright

yellow. They may coalesce into large irregular brown areas. Tan to grey tufts of fungal growth often develop on lower leaf surfaces, especially under wet and humid conditions. Infection of pods and seed can also occur. Seeds may become covered with a whitish coating of fungal hyphae and spores.

17.4.7 Frogeye Leaf Spot

17.4.7.1 Aetiology

Frogeye leaf spot is caused by the fungus *Cercospora sojina* Hara. Total 11 races of the pathogen are reported (Mian et al. 2008) and the pathogen overwinters in soybean residue and seeds. *C. sojina* is currently recognized as the causal agent of FLS, although earlier literature reported *C. daizu* as the causal agent of this disease (Athow 1987). The primary and secondary inocula are hyaline conidia of 5–7 μm × 39–70 μm, which are produced on leaf and stem residues or infested seeds.

17.4.7.2 Economic Importance

Frogeye leaf spot was first reported on soybean in Japan in 1915 (Lehman 1928) and in the United States in 1924 (Melchers 1925). Later, the disease was reported to occur in most of the soybean-growing countries. Yield reductions in the range of 10–60% due to frogeye leaf spot have been reported (Laviolette et al. 1970; Bernaux 1979; Dashiell and Akem 1991; Akem and Dashiell 1994; Ma 1994; Mian et al. 1998).

17.4.7.3 Symptomatology

Symptoms of frogeye leaf spot are most visible and typically seen on leaves but can also occur on stems, pods and seeds. Lesions on leaves begin as small, dark, water-soaked spots (Fig. 17.7). They develop into brown spots surrounded by a darker reddish-brown or purple ring. The centres of the lesions turn light brown or light grey as they age. The centre of spots may turn white with visible black specks (fungal fruiting structures) or the centres may fall away leaving a ‘shot hole’ appearance. The lesions may eventually coalesce, covering large areas of the leaves and resulting in defoliation. The disease may cause severe defoliation during warm,

Fig. 17.7 Frogeye spot

humid weather. Frogeye leaf spot can be distinguished from other soybean foliar diseases by the reddish-brown or purple ring surrounding the round leaf spots.

17.4.8 Soybean Mosaic

17.4.8.1 Aetiology

The causal agent of mosaic disease is *Soybean mosaic virus* (SMV). It has a wide host range including pea and snap bean. The virus belongs to the genus *Potyvirus*, (+)ss RNA, under the family *Potyviridae*. SMV is transmitted by infected seed, sap and soybean aphid species (*Aphis glycines*). At least 32 aphid species, belonging to 15 different genera, transmit the SMV in a non-persistent manner worldwide (Wang et al. 2006). Plants infected when young tend to show more symptoms than plants infected when old. Higher activity or populations of aphids favour virus transmission. Seed transmission rates appear to be typically below 5%, but can be higher or lower depending on the cultivar. SMV can cause yield loss, affect seed quality and reduce seed germination and nodulation. Yield reductions are generally low and infections late in the season cause little damage.

17.4.8.2 Symptomatology

Symptoms of plants infected with soybean mosaic virus can range from no apparent symptoms to severely mottled and deformed leaves. Mottling appears as light and dark green patches on individual leaves (Fig. 17.8a). Symptoms are most obvious on young, rapidly growing leaves. Infected leaf blades can become puckered along the veins and curled downward. *Soybean mosaic virus* can cause plant stunting, reduced seed size and reduced pod number per plant. The disease is one of the several factors associated with discolouration of seeds, causing a dark discolouration at the hilum (Fig. 17.8b). Symptoms of SMV may not be apparent when temperatures are above 90 °F. Symptoms are often confused with growth regulator/herbicide damage where the leaves will be elongated and which usually occurs in a pattern

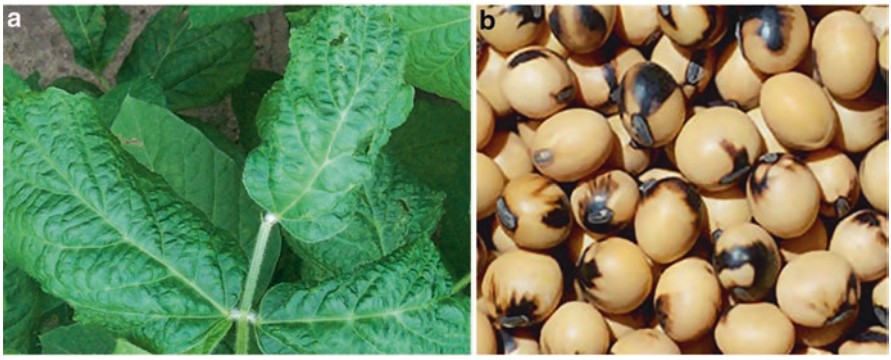

Fig. 17.8 (a) Mottled and deformed leaves and (b) dark discolouration at the hilum

such as along a field edge. SMV can interact with *Bean pod mottle virus* (BPMV) to create severe symptoms in plants infected with both viruses.

17.4.9 Pod and Stem Blight and Seed Decay

17.4.9.1 Aetiology

Pod and stem blight is caused by the fungus *Diaporthe phaseolorum* var. *sojae*. The pathogen overwinters in soybean tissue residue and in infected seeds. Host range includes green bean, pepper and tomato. Infection can occur throughout the season, although symptoms are not seen until late in the season. Wet, warm conditions and continuous monoculture of soybeans favour pod and stem blight. Insect damage or other injury to pods and seeds favours pod and seed infection. The seed decay is caused primarily by the related fungus *Phomopsis longicolla* or other species.

17.4.9.2 Symptomatology

Pod and stem blight may be more common when harvesting is delayed during wet weather. Symptoms of pod and stem blight can be confused with anthracnose, and both diseases can occur together on plants late in the season. Plants are usually infected by pod and stem blight early in the growing season. The pathogen may be present in green tissue without producing symptoms. The pathogen can infect all aerial parts of plant, but does not cause distinct lesions. Signs of infection appear on fallen petioles in mid-season and on pods and stems of plants nearing maturity. A key sign of infection is many small, black, raised dots (pycnidia) arranged in rows on infected stems, pods and fallen petioles late in the season. In wet seasons, pycnidia may cover the entire plant at maturity. Upper portions of infected plants may turn yellow and die. Infected seeds are cracked, shrivelled and dull and may have a grey mould on them. Seed infected by pod and stem blight may decompose after harvest and have low viability. Seedlings grown from infected seeds may often get blighted.

17.4.10 Stem Canker

17.4.10.1 Aetiology

Stem canker is a fairly common disease in many areas where soybeans are grown, but may not be recognized as much as it occurs. Stem canker can kill whole plants or parts of plants. It can be confused with *Phytophtora* rot. Two different kinds of stem canker are known. Northern stem canker is caused by the fungus *Diaporthe phaseolorum* var. *caulivora*. Southern stem canker is caused by the related fungus *D. phaseolorum* var. *merdionalis*.

17.4.10.2 Symptomatology

Early symptoms are reddish-brown lesions that appear at the base of branches or leaf petioles. These small lesions can develop into elongated, sunken, dark brown cankers that spread up and down the stem. Tiny black dots called perithecia (spore producing fungal structures) may appear on the stem singly or in clusters on the plants killed by

stem canker. Plant parts above the lesions may die. Reddish-brown discolouration may also occur inside the stem, and pods can abort. Leaves may develop necrosis and chlorosis between the veins and may remain attached after death. Lesions often develop at nodes and remain darker, but may extend to the soil line and create a situation easily confused with *Phytophthora* rot. These pathogens overwinter in infested soybean residue, and may spread with infected seed. Prolonged wet weather is conducive for stem canker. Reduced tillage may also favour the disease development. This disease often appears to kill or damage scattered plants, but in some cases large areas can be killed and yield losses can be significant.

17.4.11 *Septoria* Brown Spot

17.4.11.1 Aetiology

Septoria brown spot is a fungal disease caused by *Septoria glycines.* The fungus overwinters on crop debris and can spread by infected seed. In most cases, seed infection is low in commercial seed. It can be a problem in seed that has not been cleaned or has been kept for a number of years. Initial infections on primary leaves and cotyledons produce secondary inoculum that infects upper leaves as they develop. Humidity and moisture are important for brown spot development and spread (through splashing). The fungus produces a toxin that contributes to yellowing of leaves.

17.4.11.2 Economic Importance

Septoria brown spot normally does not cause major yield losses. However, losses of 5%–10% have been observed in very susceptible varieties that have been infected early and have been under prolonged stress conditions.

17.4.11.3 Symptomatology

Symptoms first appear on the primary unifoliate leaves shortly after trifoliate leaves have developed. Disease symptoms begin as small, dark brown, irregular spots in the size of 1–2 mm in diameter with or without a yellow halo, which develop on upper and lower surfaces of lower leaves. Lesions may enlarge and coalesce, and frequently they are concentrated along the leaf veins or at the leaf margin. Rapid yellowing and senescence (death) of infected leaves occurs. Symptoms may be difficult to distinguish from those of bacterial blight, soybean rust and downy mildew. One way of distinguishing the disease is the characteristic brown pycnidia (spots) found imbedded in the dead tissue of older lesions.

17.5 Seed-Borne Diseases of Rapeseed-Mustard

Rapeseed-mustard plant species are members of the family Cruciferae and mostly belongs to the genus *Brassica.* Rapeseed and mustard are mainly grown in subtropical and temperate zones of India, China, Pakistan, Canada, Poland, France, Germany and Sweden. Of the number of diseases reported on the rapeseed-mustard, important seed-borne diseases are described as below.

17.5.1 *Alternaria* Blight

17.5.1.1 Aetiology

Alternaria spp. having spores called conidia; these conidia are large and dark brown to black in colour, catenate, in chain or solitary, typically ovoid to obclavate, often beaked, multicelled and muriform (Simmons 2007). Chlamydospores and microsclerotia are reported from *A. brassicae* while *A. raphani* produces only chlamydospores. Molecular, cultural and morphological variations are reported in *A. brassicae*, although morphological variation is overlapping for identification of the *Alternaria* spp. Variation in isolates of *A. brassicae* is indicated (Mridha 1983). Studies on pathogenic variability have to be the foundation for development of prebreeding populations as strategic defence mechanism. Three distinct *A. brassicae* isolates, A (highly virulent), C (moderately virulent) and D (avirulent), are prevalent in India (Vishwanath and Kolte 1997). Start of *Alternaria* blight disease and development of the disease in epidemic form is related to weather conditions prevailing during different crop stages. It was found that severity of *Alternaria* blight on leaves (Meena et al. 2002) and pods (Sandhu et al. 1985) were higher in late sown crops. A delayed sowing results in coincidence of the vulnerable growth stage of plants with warm weather. Severity of *Alternaria* blight disease on leaves was favoured by a maximum temperature of 18–27 °C in the preceding week, minimum temperature of 8–12 °C, mean temperature >10 °C, >92% morning relative humidity (RH), >40% afternoon RH and mean RH of >70%. Disease severity on pods was positively influenced by 20–30 °C maximum temperature, >14°C mean temperature, >90% morning RH, >70% mean RH, >9 h sunshine and >10 h of leaf wetness. The regional and crop-specific models devised thereby could predict the crop age at which *Alternaria* blight first appears on the leaves and pods, at least 1 week ahead of first appearance of the disease on the crop. Under temperate conditions, the pathogen is known to survive during non-crop season on seeds and infected crop debris (Humpherson-Jones and Maude 1982). *A. brassicae* was observed to be present on seed coat and rarely in embryos of *B. compestris* var. *toria* and *B. juncea* (Shrestha et al. 2003a, b). In India, the pathogen over-winter on mustard crop grown in non-traditional areas from May to September and on the vegetable *Brassica* also during this period. *Alternaria* spores germinate on leaf surface and penetrate epithelial layer. The infection decreases the cell constituents except phenolic compounds in all the infected tissues.

17.5.1.2 Economic Importance

Alternaria blight disease has been reported from all the continents of the world and is one of the important diseases of Indian mustard causing up to 47% yield losses (Kolte 1985) with no proven source of transferable resistance in any of the hosts. Average yield losses in the range of 32–57% due to *Alternaria* blight have been reported from Nepal (Shrestha et al. 2003a, b). *Alternaria* affects most cruciferous crops, including broccoli and cauliflower (*Brassica oleracea* var. *botrytis* L.), field mustard and turnip (*B. rapa* L. (synonym, *B. campestris* L.), leaf or Chinese mustard (*B. juncea*), Chinese or celery cabbage (*B. pekinensis*), cabbage (*B. oleracea*

var. *capitata*), rape (*B. campestris*) and radish (*Raphanus sativus*). *A. brassicae* and *A. brassicicola* are cosmopolitan in their distribution. *A. raphani* and *A. alternata* are widespread in the Northern hemisphere (Jasalavich et al. 1995). Pathogens of the disease, *A. brassicicola* and *A. raphani*, are reported to cause yield losses at harvest from 5% to 15% and can reach up to 47% (Kolte et al. 1987); this loss is coupled with losses in oil quality.

17.5.1.3 Symptomatology

The pathogen produces very peculiar spots on different part of the plant starting from leaf and subsequently covering the pods and stem. Disease affects the quality of oil, yield of plant and at very start it may reduce the germination capacity of seed (Meena et al. 2010). Immediately after germination, infected plants develop black lesions on the stem of seedling that after girdling causes damage to the seedling. Most susceptible stages of crop are 45 days old seedlings and when plants are 75 days old, although symptoms start on about 40 days old seedlings (Meena et al. 2004). Different species of the pathogen *Alternaria* produces different symptoms on mustard plants. These symptoms vary with environment and host plant. *A. brassicae* produces grey colour spots on leaves, while *A. brassicicola* produces black sooty velvety spots. Distinct yellow halos are present around spots produced by *A. raphani.* Symptoms produced by *Alternaria* spp. on *Brassica* are first visible on lower leaves as small black points. These points later become large spots with concentric rings. Disease symptoms gradually spread to middle and upper leaves. Lower leaves mostly covered with *Alternaria* spots become dried and fall down. With the development of the plants, these spots appear on pods and stem, completely covering the pods and form discoloured seeds, some rotting also occur in the heavily infected pods. Due to the *Alternaria* blight disease on pods, premature plants can be seen in infected field. Mustard leaves infected with the disease have reduced photosynthetic capacity which results in yield loss as seeds remain lighter and oil quality is also affected. *Alternaria* blight disease infected plants showed decrease in triglycerides, fatty acids and different lipid classes (Atwal et al. 2003). *Alternaria* spp. produce toxins which may be present in oil extracted from *Alternaria* infected seeds.

17.5.2 White Rust/White Blisters

17.5.2.1 Aetiology

Albugo candida (Pers. ex Lev.) Kuntze is a filamentous Oomycete that belongs to the phylum Oomycota of Chromista kingdom (Agrios 2005). It is a biotrophic plant pathogen that can survive only on its host specie. Its mycelium is aseptate, intercellular with nuclei-free globular haustoria (Coffey 1975). Sporangiophores are formed beneath the epidermis, which are palisade shape and sporangia are formed in chain on sporangiophore in basipetal arrangement. Sporangia are 12–18 micron in diameter.

The sexual spores, i.e. the oospores, are developed in the stag-heads formed in the infected pods of host plant. These are thick-walled, over-wintering spores which can resist dehydration and extreme temperatures. They help tide over the unfavourable conditions and germinate in spring. The oospores present in the crop residues serve as the primary source of inoculum for the next season. The mature dehydrated zoosporangia are released by rupturing of epidermis which spread through air currents and rain droplets. These zoosporangia get rehydrated in water and germinate to release zoospores which then infect the host tissue. The germ tubes enter the stomata of resistant as readily as those of susceptible hosts. In the resistant host, mycelia growth ceases in the sub-stomatal chambers and a marked encapsulation forms around each haustorium (Verma et al. 1975).

Oospores survive in plant debris and soil, serve as primary source of infection. Oospores can remain viable for over 20 years under dry storage conditions. Possibility of survival and spread of the *Albugo* spp. by means of oospores, sporangia and mycelia on seeds have been reported (Petrie 1975; Meena et al. 2014). The optimum temperature for disease development ranged from 12 °C to 18 °C. Wetness of 3–4 h is required for disease development. Severity of white rust on leaf also needed >40% afternoon RH, >97% morning RH and 16 °C–24 °C maximum daily temperature (Chattopadhyay et al. 2011).

Physiological specialization has been noted in *A. candida* since long but their description into specialized races came late in 1963. Different races of this pathogen were classified based on their host specificity, i.e. their differential interaction on different species of Brassicaceae by Pound and Williams (1963). They identified six races of *A. candida*.

In India, 9 races of *A. candida* were identified (Singh and Bhardwaj 1984) by testing 12 *Brassica* species from 4 hosts, i.e. *B. juncea*, *B. rapa* var. *toria*, *B. campestris* var. brown sarson and *B. rapa* var. pekinensis. A detailed study to identify biological races of *A. candida* in India was conducted by Verma et al. (1999). They used a set of 14 crucifer host differentials and identified 13 races of *A. candida* in India. Of these, race 2 has higher specificity for *B. juncea* but can also infect other *Brassica* species having a common genome (Petrie 1988; Rimmer et al. 2000). Most races have been shown to infect related *Brassica* species, especially those sharing their genome with the primary host (Liu et al. 1996; Verma et al. 1999). Hence the species-specific race pathogenicity is not absolute (Verma et al. 1999). The concept of races given by Pound and Williams (1963) was based on species relationships. Contrarily, various studies have shown that cultivars of *Brassica* crops must be included in a set of host differentials to clearly define the racial status of *A. candida* (Pidskalny and Rimmer 1985; Meena et al. 2014).

17.5.2.2 Economic Importance

White rust on cultivated oilseed brassicas and other hosts have been reported worldwide. Countries where the disease occurs include the United Kingdom, the United States, Brazil, Canada, Germany, India, Japan, Pakistan, Palestine, Romania, Turkey, Fiji, New Zealand, China and Korea. White rust on sunflower occurs in Russia, Uruguay, Argentina, Australia and many other countries (Kajomchaiyakul

and Brown, 1976). White rust of salsify occurs in Australia, Canada, the United States, South America, Europe, Asia and Africa and on water spinach occurs in India, Hong Kong and also Texas (Meena et al. 2014). *A. candida* causes considerable economic losses in *Brassica* crops causing 17% to 34% yield loss (Kolte 1985). Combined infection of leaf and inflorescence causes yield losses to the extent of 62.7%, the loss being more severe (89.8%) as a result of stag-head formation in the susceptible cultivars (Lakra and Saharan 1989). Although cultural and chemical methods for control of white rust have been established, genetic resistance is the most effective and cost-efficient control of this disease.

17.5.2.3 Symptomatology

White blister disease is caused in many vegetables and *Brassica* species crops such as *B. rapa* (Chinese cabbage and turnip rape), *B. oleracea* (cabbage, kale, broccoli and cauliflower), *B. juncea* (oilseed mustard) and *B. napus* (oilseed rape) (Fan et al. 1983). Disease appears on leaves as white or creamy yellow raised pustules of about 2 mm in diameter. These pustules later coalesce and form bigger patches. Pustules are found scattered on the lower surface of the leaves (Fig. 17.9a). Corresponding upper parts of the leaf to the pustule formed on lower surface are tan yellow and form an easy recognition mark for white rust disease on plants. Completely mature pustules rupture and release white powdery mass of sporangia, which causes secondary infection. Later infected leaves start senescing with necrosis around the pustule. Pustules can also be seen on pods as a result of secondary infection. Unlike other crucifers, thickening or hypertrophy of the affected leaves is usually not seen in mustard. However, in systemic infection or infection through stem or flower, hypertrophy and hyperplasia are observed which result in the formation of stag-heads (Mundkur 1959). Affected flowers become malformed, petals become green like sepals, and stamens may be transformed to leaf-like club-shaped sterile or

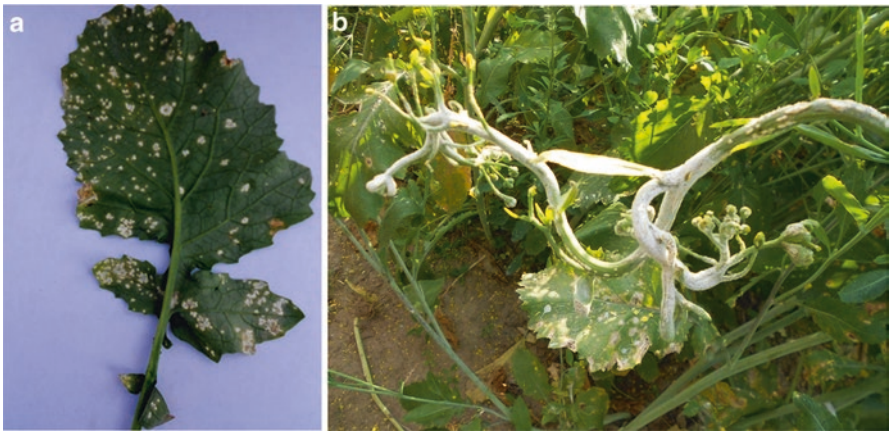

Fig. 17.9 (a) White or creamy yellow raised pustules and (b) stag-head due to hypertrophy and hyperplasia

carpelloid structures which are found to persist on the flower rather than falling early as in normal plants (Fig. 17.9b). In such conditions, ovules and pollen are atrophied leading to complete sterility. Association of downy mildew is very frequent in infected fields with the stag-head stage of white rust (Kolte 1985).

17.6 Seed-Borne Diseases of Sunflower

Sunflower (*Helianthus annuus* L.) is an important edible oilseed crop. The centre of origin of the sunflower is believed to be North America from where it has spread to Europe and Asia. Now it is grown in all continents except the Antarctica. Europe and the United States account for nearly 70% and 80% of the total production, respectively (Harter et al. 2004; Damodaran and Hegde 2007). Sunflower cultivation in Asian countries is comparatively recent. Asia accounts for nearly 20–22% of the global sunflower acerage and contributes to about 18% of the production. The productivity of sunflower in Asia is about 1.0 ton/ha, which is lower than the world average. In India, it ranks next to groundnut and soybean. The crop was introduced into India during 1969 which accounts for nearly 5% of the current oilseed production. In India, the crop is cultivated in an area of 1.48 million hectares with production of 0.9 million tonnes. Karnataka, Andhra Pradesh, Maharashtra and Tamil Nadu are the major sunflower-growing states. The crop is highly vulnerable to various diseases incited by viruses, bacteria, phytoplasma and fungi resulting in severe economic losses (Chattopadhyay et al. 2015). At least 30 diseases, caused by various fungi, bacteria and viruses, have been identified on wild or cultivated sunflower, but only a few are of economic significance as far as causing yield losses. Sunflower downy mildew, *Alternaria* blight, charcoal rot and mosaic diseases are important seed-borne diseases.

17.6.1 Downy Mildew

17.6.1.1 Aetiology

Downy mildew of sunflower is caused by an obligate fungal pathogen *Plasmopara halstedii* (Farl.) Berl. and De Toni. Sporangiophores emerge through stomata on leaves are slender, monopodially branched at right angles, bearing zoosporangia singly at the tips of branch. Zoosporangia are elliptical with an apical papilla and flattened wall. Sporangia emerge from stomata on leaves. The optimal temperature range for zoosporangia germination is 16–18 °C. This pathogen survives in seed or/and as oospore in soil debris. These oospores can survive in soil for many years. During cool and moist weather, oospores germinate and produce zoospores which infect root hairs of seedlings. For the development of disease, rain is important to predispose seedling against disease. Under field conditions where mean temperature remain 13.2 °C, seedlings remain susceptible for 15 days, if the enough rain is present during this period.

17.6.1.2 Economic Importance

Disease is found more in temperate regions where emerging seedlings are exposed to low temperature and abundant precipitation. Pathogen *Plasmopara helstedii* is considered to be of North American, Western Hemisphere, origin. The disease is present in the United States, Russia, Australia, New Zealand, Canada and European countries. Systemic infection of disease is more destructive. In France, where sunflower is grown for many years, 70–80% infection was reported. In Yugoslovakia, up to 90% yield losses have been reported (Rahim 2001; Gore 2009). Yield losses may be due to plant mortality, lighter and fewer seeds and lower oil content. The first appearance of downy mildew was reported from Oilseeds Research Station, Latur, in Marathwada region of Maharashtra State, India, where the crop is extensively grown (Mayee and Patil 1986). The disease incidence was also reported from adjoining major sunflower growing states like Andhra and Karanataka (Mayee 1988). A survey in this region conducted in 1995–1996 by Shirshikar (1997) revealed 36.67% disease incidence on farmers' fields.

17.6.1.3 Symptomatology

Sunflower downy mildew disease causes damping off and systemic infection in plant. Under cool and very wet soil conditions when seedling comes into contact with pathogen it causes damping off and seedlings are killed due to this infection. Plants infected with systemic infection remain severely stunted. Leaves of stunted plants are thick, curled downward with yellow and green epiphyllous mottling (Fig. 17.10). Downy growth of fungus appears on lower surface of the infected leaf. This shiny white cottony growth is of sporangiophores and sporangia appears on lower surface of leaves which later completely covers the lower surface. Subsequently, infected leaves become necrotic and plants remain stunted with an erect and horizontal head with very little half-filled or empty seeds (Cvjetkovic 2008). The systemic infection in the plants causes reduction in photosynthetic ability and restricted root development especially the secondary roots. Some secondary infection spots appear as angular white cottony growth on lower surface and corresponding yellowing on upper surface of leaf.

Fig. 17.10 Stunted plants with curled yellow and green epiphyllous mottling

17.6.2 Charcoal Rot

17.6.2.1 Aetiology

Disease is caused by fungus *Macrophomina phaseolina* (Tassi) Goid. It is an anamorphic fungus of *Ascomycetes*. It is usually without any sexual stage, but many times have been shown to be anamorphs with teleomorphs so they are generally grouped in *Coelomycetes*. Different isolates of *M. phaseolina* show variation in size of microsclerotia and presence and absence of pycnidia. Microsclerotia are black in colour and round to oblong or irregular in shape. Microsclerotia help in overseasoning of the fungus which is also seed-borne in nature. Pathogen is less aggressive in pre-emergence phase but more aggressive later on (Arafa et al. 2000). Mycelium is thick walled and dark brown in colour. *M. phaseolina* forms appressoria on the epidermis of sunflower and penetrates by chemical and physical means. Initial infection is through root epidermis and covers intercellular spaces which later become intracellular and distort the cells (Naz and Ashraf 2006). Infection of seed by the pathogen occurs during anthesis when seed are soft; this infection is supported by soil salinity (Fayadh et al. 2011). Moisture stress, higher temperature and period of drought favour the disease development (Alexandrov and Koteva 2001).

17.6.2.2 Economic Importance

Charcoal rot disease of sunflower is wide spread in tropical, subtropical and warm temperate regions. It is present in Latin America, Eastern and Southern Africa, Egypt, West Asia, Middle East and South Asia (Habib et al. 2007; Khan 2007; Mahmoud and Budak 2011). This disease is also reported now from America and Europe due to changing climatic conditions (Gulya et al. 2002a; Csondes et al. 2012). Crop losses are reported to the extent of 64% in Russia, 46% in India and 90% in Pakistan (Kolte 1985; Khan 2007). Increasing population density of *Macrophomina* in soil increases the yield losses in crop. It was reported that up to 79% yield losses may occur in high pathogen density soils of Pakistan (Khan et al. 2005).

17.6.2.3 Symptomatology

Under field condition wilting of plants occurs after the pollination, although the plant is infected during early stage of the growth. Symptoms of the disease appear on the stem as silver grey lesion girdling it at maturity stage. It reduces the head diameter of plant and premature death may even occur (Gulya et al. 2010; Mahmoud and Budak 2011). Lower stem becomes completely hollow due to absence of pith into this region as a result of infection. Underneath the epidermis of lower stem and on tap root, microsclerotia are observed. Pathogen's attack severely impedes the movement of micronutrient and water to upper plant parts. This results into premature wilting, progressive aging, loss of vigour and yield reduction.

17.6.3 *Alternaria* Blight

17.6.3.1 Aetiology

Alternaria leaf blight is the major disease caused by *Alternaria helianthi*. The fungus over winters as mycelium in infected plant residues and in dry conditions survives for 20 weeks in soil. The fungus is seed-borne with 22.9% seed transmissible nature. In North America, Abbas and Barrentine (1995) suggested that weeds like musk thistle and cocklebur serve as source of inoculum to infect cultivated sunflower. Conidia produced on the diseased plants serve as secondary source of inoculums for further spread of the disease in sunflower.

17.6.3.2 Economic Importance

Estimates of yield losses in sunflower due to *Alternaria* leaf blight in India ranged up to 80%. The disease reduces the head diameter, number of seeds per head, 1000 seed weight and oil content of seeds. It was reported that yield losses due to *Alternaria* blight of sunflower at two locations in Australia were 26% and 17%, respectively. The disease also affects the seed germination and vigour of seedlings. The loss in germination varies from 23% to 32%. In Northern Karnataka, Hiremath et al. (1990) reported 95% to 100% incidence of disease.

17.6.3.3 Symptomatology

The disease is a destructive one, widely distributed wherever the crop is grown. Dark brown to black, circular to oval spots varying from 0.2 to 5 cm in diameter appears on leaves. The spots are surrounded by necrotic zones with grey white necrotic centre marked with concentric rings. Spots first appear on lower leaves, later spread to middle and upper leaves. Spots enlarge and coalesce resulting in leaf blight. At later stages, spots may be formed on petioles, stem, ray florets and sometimes cause rotting of stem and heads. *A. helianthi* can cause severe leaf and stem spots resulting in premature defoliation and stem breakage, in addition to seedling blight, if infection occurs in the early stage of crop. Severe reduction in seed and oil yield reported. The most affected components are number of seeds per head and the seed yield per plant. The disease also affects the quality of sunflower seeds by affecting germination and initial vigour of the seedlings.

17.6.4 Sunflower Mosaic

17.6.4.1 Aetiology

Sunflower mosaic is caused by *Sunflower mosaic virus* (SuMV), a putative member of the family *Potyviridae*. Cells infected with SuMV had cytoplasmic inclusion bodies, typical of potyviruses. The mean length of purified particles is approximately 723 nm. The virus was transmitted by *Myzus persicae* (13%) and *Capitphorus elaegni* (20%) and also was seed-borne (12%) in sunflower cultivar Triumph 546 (Gulya et al. 2002b).

17.6.4.2 Economic Importance

First reported in *H. annuus* from Texas, USA, by Arnott and Smith (1967). Only species in *Helianthus*, *Sanvitalia* and *Zinnia*, all Asteraceae, are systemic hosts to the virus (Gulya et al. 2002b). Commercial sunflower hybrids from the United States, Europe and South Africa were all equally susceptible. Sunflower mosaic disease occurs in the United States, Argentina, the Soviet Union, Uruguay, China, India and several sunflower-growing countries of Africa.

17.6.4.3 Symptomatology

The disease is characterized by mosaic symptoms and chlorotic rings which were more severe on young leaves of plants up to 2 months of age than those of older plants. Diseased plants were stunted, sometimes had discrete narrow light green streaks on petioles and stems and produced malformed heads and shrivelled seeds.

17.7 Seed-Borne Diseases of Safflower

Safflower (*Carthamus tinctorius* L.) is one of the most important oilseed crops of the world valued for its highly nutritious edible oil. It is a multipurpose crop having various uses like source of edible oil, cattle feed, medicinal and industrial products. Safflower has good potential in dry land cropping systems as a cash crop. The total cultivated area of the safflower in the world is about 10.39 1akh hectares, and annual production is about 933 MT. The average yield per hectare is 898 Kg (Damodaran and Hegde 2007). Safflower is known to suffer from many diseases, viz. fungal leaf spot/blight (*Alternaria carthami*), wilt (*Fusarium oxysporum* f. sp. *carthami*), root rot (*Rhizoctonia bataticola*), powdery mildew (*Erysiphe cichoracearum* DC), anthracnose (*Colletotrichum capsici*), charcoal rot (*Macrophomina phaseolina* (Tassi) Goid)), bacterial leaf blight/spot (*Pseudomonas syringae* van Hall) and viral diseases (*Cucumber mosaic virus*) and necrosis (*Tobacco streak virus*) at different stages of crop growth (Jawalgaonkar 1991; Bhale et al. 1998). Despite the good agronomic performance of safflower cultivars, seed-/soil-borne pathogen particularly fungal pathogens affects directly and indirectly the quality and quantity of the oilseed crops in terms of deterioration and reduction in oil content, reduction in germination, viability of seed and potential losses in yields. As primary importance, it is the fact that seed-borne plant pathogens introduce diseases into new areas previously free from such pathogens. In safflower, *Fusarium* wilt, *Macrophomina* root rot and *Alternaria* blight are known and reported to be caused by externally or internally seed-borne fungi which are causing heavy losses to this crop.

17.7.1 *Alternaria* Blight

17.7.1.1 Aetiology

Leaf blight of safflower is caused by the fungus *Alternaria carthami* Chowdhury. At least seven species of *Alternaria* are recorded from safflower (Farr and Rossman

2015). *A. carthami* is known as a destructive disease of safflower and is recorded worldwide. It is also found on other hosts and has been reported on *Phaseolus vulgaris* in Brazil (Moraes and Menten 2006). Park and Lee (2003) also reported co-infection of *A. alternata* and *A. carthami* in safflower. Leaf blight is more severe in areas where high temperatures and high relative humidity prevail. Infected seed serves as primary source of disease; conidia produced on the diseased plants serve as secondary source of inoculums for further spread of the disease.

17.7.1.2 Economic Importance

In past years, *A. carthami* has caused up to 50% yield losses in safflower (Jackson and Berthelsen 1986). Seed infection levels of 20–55% resulted in 1.4–2.0% emergence of diseased seedlings in the field (Jackson et al. 1987). The disease apparently survived on safflower residue for at least 18 months after the infected crop had been harvested. Burns (1974) also showed that the disease survived for 2 years on safflower residue. Infected residue is an important source for disease outbreak in subsequent crops where safflower crops are often planted into the same area every second year, or in successive years. During the 1978–1979 growing season in Queensland, the disease was responsible for an estimated overall crop loss of 20%, with some crops experiencing 100% loss.

17.7.1.3 Symptomatology

The disease is seed-borne (Irwin 1976). Besides, discolouration of seeds, the fungus reduces seed quality and causes pre-emergence death, seedling death and diseased seedlings. *A. carthami* causes leaf, stem and head blight. Symptoms of large, reddish-brown, irregular lesions with yellow-green margins on leaves and flower bracts that turn white with age were recorded.

17.7.2 *Fusarium* Wilt

17.7.2.1 Aetiology

The safflower wilt caused by *Fusarium oxysporum* f. sp. *carthami* is a major disease observed in the ill-drained vertisol. Being soil-borne in nature, the fungus survives in the soil as chlamydrospores in diseased plant debris without losing viability (Chakrabarti 1979). *Fusarium oxysporum* f. sp. *carthami* invades the tap root of safflower directly or through its root hairs by mechanical means.

17.7.2.2 Economic Importance

Fusarium wilt causes more than 10% losses in yield in the safflower growing states of Maharashtra, Karnataka and Andhra Pradesh in India.

17.7.2.3 Symptomatology

An initial wilt symptom was the dropping of leaves. A characteristic symptom observed on diseased plants was the unilateral yellowing of leaves that curved towards the chlorotic side (epinasty). The yellowing of leaves started from the

lower-most leaf and progressed upwards. Stunting is evident, especially if plant infection and stress occurred during the seedling and vegetative stages. The margin of infected leaves turns tan to brown and diseased plants become progressively more yellow. Severely infected plants exhibit permanent wilting and premature defoliation. When the infection was caused during a later stage of crop growth, the lateral branches were infected on one side of the plant, while the other side apparently remained free from disease. In few cases, a white mycelium growth observed at the collar region when the wilted plants were removed from the soil. The roots did not show any external discolouration or rotting. When split open, the infected roots showed a black to brown discolouration of the vascular tissue.

17.7.3 *Macrophomina* Root Rot

17.7.3.1 Aetiology

Macrophomina phaseolina.

17.7.3.2 Economic Importance

It is a serious menace in Karnataka, India and may kill up to 20–25% of plants in commercial fields (Singh and Bhowmikh 1979).

17.7.3.3 Symptomatology

M. phaseolina infected plants show poor growth and reduced size of inflorescences. Stem-split was observed in 30 days old plants as minute cracks approximately 2–3 cm above the soil surface, which over time extended to both upward and downward directions, resulting in the formation of a wider split. The split portion was hollow and brown with a white to grey mycelial mat of the fungus on the inner surface. Such plants lodged, ultimately resulting in poor seed yield compared with healthy plants. The stem-split plants showed delayed flowering by 1 week over healthy plants.

17.8 Conclusion

Seed-borne diseases of edible oilseed crops are potential threat to its cultivation as these crops are grown in resource-poor agricultural lands. Due to difficulty in detection of seed-borne diseases and non-availability of quality seeds free from seed-borne pathogens, edible oilseed crops' cultivation becomes week in India. Strict practices, viz. rejection of diseased seed production fields, increasing crop health monitoring and compulsory seed treatment practices, will boost the edible oilseed cultivation.

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Major Seed-Borne Diseases in Important Pulses: Symptomatology, Aetiology and Economic Importance

18

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Abstract

Pulses are an important accompaniment in the diet along with staples like rice and wheat. They are very important for food security and more importantly nutrition security particularly for large vegetarian population, where the major sources of protein are vegetable sources. Pulses contain other essential nutrients that help the body fight vitamin and mineral deficiencies and diseases. Additionally, pulses contribute to soil fertility due to their nitrogen-fixing ability, thus reducing usage of chemical fertilizers in subsequent crops. Realizing the importance of pulses in the human diet, 2016 has been declared the International Year of Pulses (IYP). Pulse crops suffer losses due to diseases, insect pests, drought, waterlogging, salinity and a variety of other stress factors. Thus, several factors are responsible for low production of pulses. Seed is the first source of inoculum of pathogens and its transmission. 'Pathogen-free' or 'disease-free' or 'zero-tolerance' concept becomes practically an unrealistic and impossible goal under these conditions. Among the diseases of pulses, seed-borne/seed-transmitted diseases play a crucial role in the reduction of achievable crop yields. The major seed-borne diseases of pulses are *Ascochyta* blight, *Botrytis* (grey) mould, *Stemphylium* blight, anthracnose, *Alternaria* leaf spots, bacterial blight, mosaic, etc. In consideration of all these factors, the important seed-borne diseases of major pulses and their management are discussed in this chapter.

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18.1 Introduction

Pulses are an important crop group in the cropping systems of several developing countries in Asia, Africa and Latin America. Pulses in these regions are an important component of the diet along with staples like rice and wheat. They are of particular importance for food security and more importantly nutrition security particularly in low-income countries, where the major sources of protein are pulses and vegetables. Protein content of pulses, averaging about 20–25%, is higher than cereals, which average 5–10%. In addition, pulses contain other essential nutrients such as calcium, iron and lysine that help the body fight vitamin and mineral deficiencies and diseases. As on the consumer's plate, so too in the production system: pulses on average occupy only about 10–15% of the arable land area but contribute significantly to the value of agricultural production due to their higher prices. Additionally, pulses also contribute to soil fertility due to their nitrogen-fixing ability, thus reducing usage of chemical fertilizers in subsequent crops. Due to the growing awareness on the nutritional value of pulses and a move towards vegetarianism, since two to three decades back, developed countries (particularly North America) are also using pulses for their domestic food; earlier it was produced there mainly for livestock feed and export to developing countries. Thus pulse crops can potentially help improve health and nutrition, reduce poverty and hunger and enhance ecosystem resilience. Realizing the importance of pulses in the human diet, 2016 has been declared the International Year of Pulses (IYP). At the global level, the average share of pulses is only 5% of the total protein consumption, but their contribution in several developing countries ranges between 10% and 40%. To meet the growing demand and raise their per capita availability, countries made efforts to increase production and explore trade opportunities to augment domestic supply.

According to Calles (2016), only legumes harvested for dry grain are classified as pulses. Legume species used for oil extraction [e.g. soybean (*Glycine max* (L) Merr) and groundnut (*Arachis hypogaea* L)] and forage purposes [e.g. clover (*Trifolium* spp.) and alfalfa (*Medicago sativa* L)] are not considered pulses. Likewise, legume species are not considered as pulses when they are used as vegetables [e.g. green peas (*Pisum sativum* L) and green beans (*Phaseolus vulgaris* L)]. Thus, when common bean (*Phaseolus vulgaris* L) is harvested for dry grain, it is considered as a pulse; but when the same species is harvested unripe (known as green beans), it is not treated as a pulse. According to FAO (1994), the classification of plants that are considered to be pulses are Common bean *P. vulgaris* L, Lima bean *P. lunatus* L, Scarlet runner bean *P. coccineus* L, Tepary bean *Phaseolus acutifolius* A Gray, Adzuki bean *Vigna angularis* (Willd) Ohwi & H. Ohashi, Mung bean *Vigna radiata* (L) R Wilczek, Mungo bean *Vigna mungo* (L) Hepper, Rice bean *Vigna umbellata* (Thunb) Ohwi & H Ohashi, Moth bean *Vigna aconitifolia* (Jacq) Maréchal, Bambara bean *Vigna subterranea* (L) Verdc, Broad bean *Vicia faba* L, Common vetch *Vicia sativa* L, Pea *P. sativum* L, Chickpea *Cicer arietinum* L, Cowpea *Vigna unguiculata* (L) Walp, Pigeon pea *Cajanus cajan* (L) Huth, Lentil *Lens culinaris* Medik, Lupines *Lupinus* species, Hyacinth bean *Lablab purpureus* (L), Sweet Jack bean *Canavalia ensiformis* (L) DC, Winged bean *Psophocarpus*

tetragonolobus (L) DC, Guar bean *Cyamopsis tetragonoloba* (L) Taub, Velvet bean *Mucuna pruriens* (L) DC and African yam bean *Sphenostylis stenocarpa* (Hochst ex A Rich) Harms. Legume crops grown in warm season in India and Southeast Asia (Pandey et al. 2009) include blackgram (*Vigna mungo* (L.) Hepper), greengram (*Vigna radiata* (L.) Wilczek), kulthi (*Macrotyloma uniflorum* (Lam.) Verdc.) and pigeonpea (*Cajanus cajan* (L.) Millsp.), whereas legumes grown in cool season are fieldpea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.) and khesari (*Lathyrus sativus* L.).

Several explanations for the low production of pulses have been offered. Most of the pulse crops are grown by small farmers either on marginal lands and as intercrops or as catch crops on residual moisture (only 9% of the area under pulses is irrigated). The use of inputs particularly improved seeds and chemicals is scanty. Pulse crops suffer losses due to diseases, insect pests, drought, waterlogging, salinity and a variety of other stress factors. Support prices and marketing arrangements are inadequate. Risks are twofold; biotic and abiotic stress factors result in low production and fluctuating prices. Diseases of pulse crops are serious yield reducers and contribute substantially to instability of production (Nene 1986). The major obstacles for increase in production are undoubtedly the diseases which cause losses to the tune of 20–30%. Different pulses suffer from various fungal, viral, bacterial and nematode diseases. Notable examples are wilt, *Ascochyta* blight and grey mould of chickpea; sterility mosaic, *Phytophthora* blight and wilt of pigeonpea; yellow mosaic, *Cercospora* leaf spots and *Macrophomina* blight of *Vigna* species; powdery mildew of pea; downy mildew of Lathyrus; as well as bacterial diseases of green gram, cowpea and pigeon pea. Among nematode diseases, cyst nematode of pigeonpea, root knot of chickpea and *Rotylenchus* cause serious damage to various pulse crops. High yielding varieties with multiple resistances to major diseases should be identified, adopted and popularized for better disease management. Seed is the richest source to be attracted by pests and diseases resulting into several seed-borne diseases. An effective as well as cheap technology is to treat the seed with some suitable fungicide like bavistin, captan or thiram at the rate of 2.5 g/kg of seed. Such a treatment is essential to bring down seed-borne and soil-borne fungi in early stages of crop growth (Pal 1996).

Since many plant pathogens can survive in, on, or with seed, seed has been and continues to be an important vehicle for transmitting plant pathogens throughout the world; the use of clean seed is an important disease control measure (Kuan 1988). For example, in 1984 P. Hewett reported at the 18th International Seed Testing Association, Plant Disease Committee (ISTA-PDC) Workshop that the seed of *Vicia faba* carried three viruses and *Ascochyta* leaf and pod spot from North Africa, Belgium and Germany to England and then from England to France, Denmark, Canada, New Zealand and Australia. Many plant viruses occur widely in plant germ plasm collections, and these viruses can be transmitted through exchange and use of germ plasm. For example, *Soybean mosaic virus* has been distributed through the world via international exchange of soybean germ plasm. This virus in *Glycine max* germ plasm collections has reduced yield of affected germ plasm accessions (Goodman and Oard 1980). The outbreak of *Pea seed-borne mosaic virus* in the

1970s has been traced back to *Pisum sativum* germ plasm (Hampton and Braverman 1979). Thus, the use and exchange of clean seed are an important method to manage plant disease. Many public and private institutes (Kuan 1983) involved with seed exchange activities realize this importance and are using every possible means to produce and ship clean seed. Some important pulse crops with their major seed-borne diseases are described below.

18.2 Nutritional Benefits of Pulses

Pulses are an excellent complementary food for infants and young children to meet their daily nutritional needs. They can be incorporated into children's diets through family diets and school meal programmes in both developed and developing countries. Their high nutrient content also makes pulses ideal for vegetarians and vegans to ensure adequate intakes of protein, minerals and vitamins (Tables 18.1, 18.2 and 18.3). When combined with food high in vitamin C, pulses' high iron content makes them a potent food for replenishing iron stores, particularly for women at reproductive age, who are more at risk for iron deficiency anaemia. Older people can also benefit from eating pulses. In many cultures, pulses are considered as 'protein for the poor'. There are a number of reasons why they are underestimated. The most common ones are: they can cause bloating, flatulence, and, unless they are soaked for hours, pulses take a long time to cook. Pulses contain some anti-nutrients, which are substances that reduce the body's ability to absorb the various minerals that pulses contain. Fortunately, many of these issues (bloating, flatulence, anti-nutrients and length of cooking time) can be overcome using traditional cooking techniques, such as soaking, germination (sprouting), fermentation and pounding. Traditional methods can also help to reduce the content of the anti-nutrients. When other foods are combined with pulses, the nutritional value of pulses is further enhanced, as other foods help to ensure that the body is able to better absorb all the nutrients found in pulses (FAO 2016).

18.3 Chickpea (*Cicer arietinum* L.)

Chickpea is one of the most important edible food legumes in the world after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). It is a cool season food crop mostly grown in dry lands. The crop suffers from serious diseases that affect it in all growth stages. The pathogens that affect chickpea include fungi, bacteria, viruses, nematodes and mycoplasma, which result in severe economic losses globally. Among these, fungi are the largest group of the pathogens. Nearly, 172 pathogens have been reported so far that infect chickpea (*Cicer arietinum* L.) in different parts of the world (Nene et al. 1996). Out of these, some diseases are persistent problems in chickpea production in wide geographical areas, notably, *Ascochyta* blight, *Fusarium* wilt, dry root rot, stunt (caused by *Bean (pea) leaf roll virus*), *Botrytis* grey mould, collar rot, black root rot, *Phytophthora* root rot and

Table 18.1 Nutritive value of pulses (Gopalan et al. 2004)

Pulses	Energy (Kcals)	Moisture (g)	Protein (g)	Fat (g)	Mineral (g)	Carbohydrates (g)	Fibre (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)
Bengal gram, whole	360	10	17	5	3	61	4	202	312	5
Bengal gram, *dhal*	372	10	21	6	3	60	1	56	331	5
Bengal gram, roasted	369	11	22	5	2	58	1	58	340	9
Black gram, *dhal*	347	11	24	1	3	60	1	154	385	4
Cowpea	323	13	24	1	3	54	3	77	414	9
Field bean, dry	347	10	25	1	3	60	1	60	433	3
Green gram, whole	334	10	24	1	3	57	4	124	326	4
Green gram *dhal*	348	10	24	1	3	60	1	75	405	4
Horse gram, whole	321	12	22	0	3	57	5	287	311	7
Khesari, *dhal*	345	10	28	1	2	57	2	90	317	6
Lentil	343	12	25	1	2	59	1	69	293	7
Moth beans	330	11	24	1	3	56	4	202	230	9
Peas green	93	73	7	0	1	16	4	20	139	1
Peas dry	315	16	20	1	2	56	4	75	298	7
Peas roasted	340	10	23	1	2	57	4	81	345	6
Rajmah	346	12	23	1	3	61	5	260	410	5
Redgram, *dhal*	335	13	22	2	3	58	1	73	304	2
Redgram tender	116	65	10	1	1	17	6	57	164	1
Soyabean	432	8	43	20	5	21	4	240	690	10

Table 18.2 Dietary fibre in pulses and legume foods (g/100 g) (Narasinga Rao 2002)

Foods	Total	Non-cellulose polysaccharides			Cellulose	Lignin
		Total	Water soluble	Water insoluble		
Legumes with husk						
Bengal gram	26.0	10.1	3.7	6.4	13.4	2.5
Soya bean	20.8	9.9	34	6.5	8.2	2.7
Winged bean	35.2	17.0	9.7	7.3	13.3	4.9
Kesari *dhal*	18.6	6.4	2.6	3.8	9.1	3.1
Dhals						
Red gram	13.4	10.3	–	–	2.1	1.0
Black gram	13.6	9.5	–	–	2.9	0.9
Bengal gram	13.4	7.1	–	–	5.5	1.2
Green gram	13.2	8.6	–	–	3.7	1.0
Rice	8.3	4.1	–	–	2.7	1.6
Wheat	17.2	106	–	–	5.5	1.2
400 g rice +75 g black gram *dhal*	43.4	23.5	–	–	13.0	7.1
400 g wheat +50 g black gram *dhal*	75.6	49.2	–	–	23.5	5.3

Table 18.3 Isoflavone content of pulses and legumes (mg/100 g fresh weight) (Narasinga Rao 2002)

Isoflavones	Soya bean	Bengal gram	Green gram	Black gram	Red gram
Daidzein	2.7 (49)	5.1	80.7 (81)	88.0 (98.0)	85[a]
Protensin	–	4.8	–	–	12
Formononetin	–	44.1	–	–	–
Biochinin A	–	98.6	–	–	–
Genistin	95.4	–	–	–	–
Genistein	2.4	–	–	–	–
Daidzin	40.6	–	–	–	–
Total	67.8–388.6	152.6	80.7	88.8	97

[a]Content on sprouting

Pythium root and seed rot, while others are sporadic in occurrence or endemic in distribution. Diseases with limited distribution may still be economically important locally. Due to continuous changes in cultural practices, human interventions and climate change, some of the minor diseases may become economically important. One such example is dry root rot (*Rhizoctonia bataticola*) of chickpea, which is emerging as a potential threat to chickpea cultivation in semi-arid regions because the host plant is predisposed to infection by moisture stress and high temperatures during the flowering to pod filling stage (Sharma et al. 2010). *Fusarium* wilt and *Ascochyta* blight are serious diseases, which are of great economic importance causing significant yield losses (Nene et al. 2012).

18.3.1 *Ascochyta* Blight

18.3.1.1 Distribution and Economic Importance

It is one of the important biotic constraints for chickpea production and causes significant loss of grain yield and quality (Gaur and Singh 1996). Cool and wet weather conditions favour the disease development and often result in 100% yield loss (Reddy et al. 1990; Singh et al. 1992; Singh and Reddy 1993). The disease spreads by airborne spores and also by infected seeds. Fungicidal treatments to control the disease are often impractical and uneconomical (Reddy et al. 1990). *Ascochyta* blight was first identified in the then North-western Province of India, presently in Pakistan. It has been reported in 37 countries viz., Bangladesh, China, India, Iran, Iraq, Israel, Jordan, Lebanon, Libya, Pakistan, Syria, Turkey, Algeria, Cyprus, Egypt, Ethiopia, Morocco, Sudan, Tanzania, Tunisia, Bulgaria, France, Greece, Hungary, Italy, Portugal, Romania, Spain, Volga, Northern, Caucasia, Canada, the United States, Columbia, Mexico and Australia. The economic importance of the disease is evident from the frequent occurrence of epidemics causing serious losses in many countries. In 1981–1983, the chickpea crop was completely destroyed in the north-western states of India and Pakistan, and the latter was compelled to import pulses worth $7.43 million (Verma et al. 1981; Singh et al. 1982a, b). Substantial losses were also reported from the Mediterranean and up to 100% in Italy and Spain (Hawtin and Singh 1984). In the Pacific North-west, a severe epidemic of *Ascochyta* blight caused financial loss of more than $1 million in 1987 (Kaiser and Muehlbauer 1988). The 1998 Australian epidemic (Galloway and MacLeod 2003) devastated the industry and caused a shift in production from 105,000 ha in 1998 to 15,000 ha in 1999 in a single state (Moore et al. 2004). Severe crop losses and epidemics of the disease have been reported by several workers (Zalpoor 1963; Radulescu et al. 1971; Kaiser 1972; Nene 1984) and Pande et al. (2005a) gave a detailed review of this disease.

18.3.1.2 Symptomatology

The disease attacks all above-ground parts of chickpea plants at all growth stages, causing necrotic lesions on leaves, stems and pods (Fig. 18.1). Depending on varietal susceptibility, temperature and humidity, lesions appear 4–11 days after infection and are usually circular on leaves and pods, and oval on stems and brown or dark brown in colour. A characteristic feature of *Ascochyta* blight is the formation of many black specks (pycnidia) in the lesions, usually in concentric circles (Fig. 18.1). Infected stems often collapse and break off. Pod infection may result in seed infection or contamination (Singh et al. 2007). The disease is usually seen around flowering and podding time as patches of blighted plants in the field (Fig. 18.1). Symptoms are seen on all above-ground parts of the plant. However, the disease can appear at a very early stage of crop growth. When the pathogen is seed-borne and conditions at the time of germination are conducive to disease development, the emerging seedlings develop dark brown lesions at the base of the stem. Affected seedlings may collapse and die (damping-off) (Fig. 18.1). Pycnidia may be formed on the lesions (Fig. 18.1). Isolated infected seedlings may not be noticed.

Fig. 18.1 *Ascochyta* blight of chickpea

But at flowering and podding time, when conditions are usually favourable for disease development, the disease spreads from these isolated seedlings, resulting in patches of blighted plants. When the source of inoculum is air-borne conidia or ascospores, the disease initially appears in the form of several small water-soaked necrotic spots on the younger leaves of almost all branches. These spots enlarge rapidly and coalesce, blighting the leaves and buds under favourable conditions. Pycnidia are observed on the blighted parts. On a susceptible cultivar, the necrosis progresses from the buds downwards, killing the plant. In cases of severe foliar infection, the entire plant dries up suddenly. If conditions are not favourable for disease development (hot dry weather), the plants do not die and the infection remains in the form of discrete lesions on the leaves, petioles, stems and pods. The symptoms on the leaflets are round spots with brown margins and a grey centre that contains pycnidia, which are often arranged in concentric rings. On the stems and petioles, the lesions are obovate or elongate and bear pycnidia. The size of the lesions varies greatly; some may become 3–4 cm long on stems and often girdle the affected portion. The stems and petioles usually break at the point of girdling. If blight occurs at the preflowering stage and then conditions for its development become unfavourable (hot dry weather), the crop regrows fast but symptoms can still be seen on the older branches (Fig. 18.1). Fully developed lesions on pods are usually round, up to 0.5 cm in diameter, usually with concentric rings of pycnidia. Several lesions may appear on a single pod and if infection occurs in the early stages of pod development, the pod is blighted and fails to develop any seed. Late infections result in shrivelled and infected seed. The fungus penetrates the pod and infects the developing seed. Symptoms on the seeds appear as a brown discolouration and often develop into deep, round or irregular cankers, sometimes bearing pycnidia visible to the naked eye (Nene et al. 2012).

18.3.1.3 Aetiology

Ascochyta blight of chickpea is caused by the fungus *Ascochyta rabiei* (Pass.) Lab. (Teleomorph: *Didymella rabiei* (Kovachevski) vs Ark). The pathogen produces its asexual stage as minute dot-like black fruiting bodies (pycnidia) in concentric rings. The pycnidia contain numerous hyaline conidia. The sexual stage was first reported by Kovachevski (1936) from Bulgaria and has subsequently been found in most countries where the disease occurs (Trapero-Casas and Kaiser 1992), the most recent being Australia (Galloway and MacLeod 2003; Galloway et al. 2005). It is a seed-borne disease. Diseased debris left over in the fields also serves as a source of primary inoculum. Ascospores were also found to play a role in the initiation of disease epidemics. Secondary spread is through pycnidiospores. Chickpea and its wild relatives are the only confirmed hosts of the fungus *A. rabiei*. However, Kaiser (1990) reported other hosts of the pathogen, outside the genus *Cicer*. Cool, cloudy and wet weather favours the disease development. The disease builds up and spreads fast when night temperatures are around 10 °C, day temperatures are around 20 °C and rains are accompanied by cloudy days. Excessive canopy development also favours blight development.

18.3.1.4 Management

Management is essential to provide increased and stable chickpea yields throughout the world. Where possible, HPR should be emphasized over chemical control as the most environment-friendly and economic disease control strategy. Selection of resistant sources for genetic improvement programs should be based on resistance to disease at vegetative, flowering and podding stages, since many lines resistant in the vegetative stage can be susceptible at the podding stage. Resistance to *Ascochyta* blight in chickpea cultivars has historically been overcome by new pathotypes of *A. rabiei*; hence the genotypes intended for release to farmers should be selected based on multi-location multi-season field trials. Durable resistance may only be possible if an array of resistance genes is combined providing different mechanisms of resistance against all races in a single cultivar. Studies are underway to determine the genetics and allelic relationships of resistance to *Ascochyta* blight in different genotypes as an essential precursor to pyramid resistance genes. Knowledge of the variability of *A. rabiei* is also a prerequisite for breeding programs aimed at obtaining durable resistance to *Ascochyta* blight. Further studies on the ecology of *A. rabiei* and its epidemiology are required to improve the current disease management strategies. Both innovative and conventional approaches should be used to investigate the host–pathogen relationship between *C. arietinum* and *A. rabiei*, and to develop better methods for resistance screening. Development of marker-assisted selection methods will enable rapid screening of different genotypes and breeding populations for disease resistance. Moreover, pyramiding of different sources and/or mechanisms of resistance sharing a similar phenotype will only be possible through the application of molecular breeding tools (Pande et al. 2005a). A number of cultural practices are available in managing *Ascochyta* blight. These include planting resistant cultivars, use of disease-free seed, crop rotation with cereal or other non-host crops, deep ploughing, sanitation and intercropping

with wheat, barley and mustard to reduce the disease spread. Delayed planting, where practical, also helps escape or reduce the initial primary inoculum source from forcibly discharged air-borne ascospores, as does isolating current season crops from paddocks sown previously to chickpea. Chemical control of *Ascochyta* blight is through both seed treatment and foliar spray. The primary seed-borne infection can easily be controlled by treating the seed with calixin-M (11%) tridemorph (36% Thiram), Bavistin + Thiram (1:1), Hexacap, Captan, Captafol, Thiabendazole + Thiram and Rovral. Seed treatment also enhances seed germination (Singh and Singh 1990). The secondary spread of the disease can be controlled by timely application of foliar fungicides. In India, complete control of the disease can be achieved by treating the seed with any of the above fungicides and applying 2–3 sprays of Hexacap, Captan, Captaf, Indofil M-45 or Kavach. In Australia, chlorothalonil was more effective than Mancozeb both as a preventative and salvage treatment, providing application takes place before the rains (Moore et al. 2006). The most effective practices include using a combination of disease-free seed, destruction or avoidance of inoculum sources, manipulation of sowing dates, seed and foliar fungicides and cultivars with improved resistance (Davidson and Kimber 2007). For *Ascochyta*-susceptible chickpeas, the use of disease-free seed, or seed treatments, is crucial as seed-borne infection is highly effective as primary inoculum and epidemics develop rapidly from foci under favourable conditions. Implemented fungicide strategies differ according to cultivar resistance and the control efficacy of fungicides, and the effectiveness of genetic resistance varies according to seasonal conditions. Studies are being undertaken to develop advanced decision support tools to assist growers in making more informed decisions regarding fungicide and agronomic practices for disease control (Davidson and Kimber 2007).

18.3.2 *Botrytis* Grey Mould

18.3.2.1 Distribution and Economic Importance

Botrytis grey mould (BGM) is a devastating disease of chickpea in South Asian countries like India, Bangladesh, Nepal and Pakistan and seriously damages crops in other countries like Australia, Argentina, Myanmar, Canada, Columbia, Hungary, Mexico, Spain, Turkey, the United States and Vietnam. In India, BGM appeared as an epidemic in 1968 on the chickpea crop in Tarai area extending to the submontane region of Uttar Pradesh (Joshi and Singh 1969) and later on, during 1981–1983, the disease occurred along with *Ascochyta* blight in the north-western states of India, such as Punjab, Haryana, Himachal Pradesh, Rajasthan, parts of Bihar and West Bengal causing 70–100% yield loss (Singh et al. 1982a, b; Grewal and Laha 1983). The disease frequently causes total yield loss in the Indo-Gangetic Plains (IGP) of India, Nepal and Bangladesh (Singh and Sharma 2002; Pande et al. 2005b). Losses up to 96% have also been reported from Argentina (Carranza 1965).

Fig. 18.2 *Botrytis* grey mould of chickpea

18.3.2.2 Symptomatology

Lack of pod setting is the first indication of the disease. Leaves and stems may not show any symptoms. Under highly favourable weather conditions, foliage shows clear symptoms and plants often die in patches (Fig. 18.2). The disease is more severe on plant parts hidden under the canopy and is obvious if the canopy is parted to expose the symptoms. Shed flowers and leaves covered with the spore mass can be seen on the ground under the plants. When humidity is very high, the symptoms appear on stems, leaves, flowers and pods as grey or dark brown lesions covered with mouldy growth. Lesions on stem are 10–30 mm long and girdle the stem completely. Tender branches break off at the point where the grey mould has caused rotting (Fig. 18.2). Affected leaves and flowers turn into a rotting mass. Lesions on the pod are water-soaked and irregular (Fig. 18.2). On infected plants, the pods contain either small, shrivelled seeds or no seeds at all (Fig. 18.2). Greyish white mycelium may be seen on the infected seeds (Fig. 18.2) (Nene et al. 2012).

18.3.2.3 Aetiology

BGM is caused by *Botrytis cinerea* Pers. Ex Fr. (Teleomorph: *Botryotinia fuckeliana* Grover and Loveland). It is a seed-borne disease. The fungus has a very wide host range. The disease is usually seen at flowering stage when the crop canopy is fully developed. Excessive vegetative growth due to too much irrigation or rain, close spacing and varieties that have a spreading habit favour disease development. Temperatures between 20 and 25 °C and excessive humidity around flowering and podding stage favour disease development. As temperatures favourable to BGM are slightly higher than those for *Ascochyta* blight, these diseases may occur one after the other with *Ascochyta* blight appearing first. Initial fungal growth is white and cottony and turns grey with age. The conidia are smooth, hyaline to pale brown, ellipsoidal, one-celled. Conidiophores are tall and dark bearing short branches with

terminal ampulla on their apex on which clusters of conidia are formed on short denticles. The teleomorphic state of this fungus has been reported from sclerotia of *B. cinerea* on chickpea in India. The disease can appear at any growth stage, but the maximum development of the disease takes place at reproductive stage. It attacks flowers, pods, leaves and stems, of which the flowers are more vulnerable. Initial symptoms under artificial conditions are water soaking and softening of affected plant parts. On these parts, brown spots are produced, which are rapidly covered with dense sporophore mass of conidia and mycelium. Under field conditions such grey fungal growth can be seen on flowers, pods and stems hidden under a dense canopy in wet conditions. On stem, BGM symptoms are gradually replaced by dark grey to black sporodochia. When relative humidity is low, irregular brown spots without any fungal growth appear on leaves. Sometimes, small, tiny black sclerotia are produced on dead tissues and water-soaked lesions on pods. The affected pods either do not produce any seed or produce small and shrivelled seeds (Singh and Sharma 2002). *B. cinerea* can survive through infected seeds, crop debris and other host plants either parasitically or saprophytically. The infected seeds carry the pathogen from season to season, to new areas, which were earlier known to be disease free. It can survive on seeds externally as well as internally up to 5 years at a temperature of 18 °C (Pande et al. 2005b). The pathogen can also survive in the soil in the form of mycelium and sclerotia. Small infected plant debris mixed in the seed also plays an important role in the survival of the pathogen. *B. cinerea* is a facultative parasite having a wide host range. It infects several plant species such as vegetables, fruits, ornamental plants, legumes and several weed hosts (Farr et al. 2006). Severe epidemics of BGM occur frequently in many South Asian countries. Free moisture, high relative humidity and temperature ~20–25 °C are congenial for the infection and development of the disease. *B. cinerea* requires a wet period of 6 h, incubation period of 36 h and latent period of 72 h (Singh and Kapoor 1985). The pathogen can complete the entire disease cycle in 7 days under favourable conditions. The pathogen can attack the basal parts of the plant early in the season and move from the seed to epicotyl portion. A closed chickpea canopy with reduced light penetration and air movement creates ideal conditions for fast spread of the disease. Under such conditions, there is abundant sporulation of the fungus on dead plant parts, particularly, on flowers and pods (Pande et al. 2005b).

18.3.2.4 Management

Late planting, lower seed rate and increased plant spacing often help to avoid or reduce the disease. New chickpea varieties with moderate type of resistance to BGM are also available. Chemical control has been effective either as seed treatment or as foliar spray. Repeated spray is necessary if disease-conducive conditions persist. The seed-borne infection can be completely eliminated by treating the seed with a combination of Bavistin + Thiram (1:1). Effective fungicides include Indofil M 45, Bavistin, Thiabendazole, Rovral and Ronilan (Singh et al. 2007).

18.3.3 *Fusarium* Wilt

18.3.3.1 Distribution and Economic Importance

Chickpea wilt is a very important disease and occurs in 32 countries falling in the six continents (Nene et al. 1991; Singh and Sharma 2002). The pathogen in association with other soil-borne pathogens like root rots and foot rot also causes extensive damage to chickpea crop. It causes around 10% yield loss in India but under certain conditions and specific locations, the losses may go up to 60%. The disease is becoming a major constraint in chickpea production in California, USA, and the Mediterranean (Haware 1990). Yearly yield losses are estimated at 10–15% in India and Spain, with losses of 70–100% in years of severe outbreaks of the disease (Nene et al. 2012).

18.3.3.2 Symptomatology

The characteristic symptoms of wilt are drooping of petioles, rachis and leaflets (Fig. 18.3). The lower leaves are chlorotic, gradually turn yellow and then light brown or straw-coloured and finally dry up. Discolouration of xylem vessels extends towards stems and branches and can be seen when split open vertically (Fig. 18.3). Sometimes only a few branches are affected, resulting in partial wilt. Affected plants do not show external root discolouration. However, coinfection with other soil-borne pathogens may cause external root discolouration (Singh et al. 2007). The field symptoms of wilt are dead seedlings or adult plants, usually in patches (Fig. 18.3). The disease can affect the crop at any stage. At seedling stage, whole seedlings (3–5 weeks after sowing) collapse and topple down on the ground. These seedlings retain their dull green colour. When uprooted, they usually show uneven shrinking of the stem above and below the collar region (soil level) (Fig. 18.3). The shrunken portion may be about 2.5 cm or longer. Affected seedlings do not rot at stem or root surface. However, when split open vertically from the collar

Fig. 18.3 *Fusarium* wilt of chickpea

downwards or cut transversely, dark brown to black discolouration of the internal stem tissues is clearly visible. In seedlings of highly susceptible cultivars, the black discolouration may not be clearly visible. However, internal browning from root tip upwards is clearly seen. The affected plants show typical wilting, i.e. drooping of the petioles, rachis and leaflets. Drooping is visible initially in the upper part of the plant but within a day or two, the entire plant droops. The lower leaves are chlorotic, but most of the other leaves droop while still green. Gradually, however, all the leaves turn yellow and then light brown or straw coloured. Dried leaflets of infected plants do not shed at maturity (Fig. 18.3). Affected plants, when uprooted and examined before they are completely dry, show no external rotting, drying or root discolouration. When the stem is split vertically, internal discolouration can be seen. Around the collar region, above and below, the xylem in the central inner portion (pith and part of the wood) is discoloured dark brown or black. In the initial stage of wilting, the discolouration may not be continuous. *Discolouration* also extends several centimetres above the collar region into the main stem and branches. Sometimes only a few branches are affected, resulting in partial wilt. In certain cultivars, the lower leaves dry up before the plants wilt (Nene et al. 2012).

18.3.3.3 Aetiology

This disease is caused by *Fusarium oxysporum* Schlechtend. emend. Snyd. et Hans. f. sp. *ciceri* (Padwick) Matuo et K. Sato. It produces microconidia, macroconidia and chlamydospores. Microconidia are 2.5–4.5 × 5–11 μ in size, oval or cylindrical, straight or curved. Macroconidia are 3.5–4.5 × 25–65 μ in size, septate or fusoid. Both microconidia and macroconidia are generally sparse on solid media and are formed abundantly in potato dextrose broth. Chlamydospores are formed in 15-day-old cultures singly, in pairs or in a chain, and are smooth or roughwalled. Best growth of the fungus can be obtained at 25 °C and pH 6.0. The disease appears around 20 days after sowing and even earlier on susceptible cultivars. The pathogen is both soil- and seed-borne, and its infection is systemic. It can be isolated from the aerial plant parts, including seeds. The pathogen can survive on infected crop residues buried in the soil for up to 6 years. The infected seeds play an important role in spread of the disease to disease-free areas. Other leguminous host plants such as lentil, pea, pigeon pea, bean and faba bean have been identified as symptomless carriers. The pathogen can survive through mycelium and chlamydospores in seed and soil (Haware and Nene 1982). The epidemiology of root-infecting fungi in the soil is complex and involves several factors. Environmental and physical factors such as soil moisture, soil temperature, soil nutrients, pH, inoculum density, race, plant age and host genotype play an important role in the development of the disease (Singh and Sharma 2002). Light and sandy soils, alkaline soils, soil moisture and temperature around 25 °C favour development of the disease (Chauhan 1963). Wilt incidence is generally higher when chickpea is grown in warmer and drier climates (>25 °C) and when crop rotations are not practiced (Nene et al. 2012).

18.3.3.4 Management

Collection of diseased plant debris and deep ploughing after harvest can reduce the inoculum and consequently reduce disease incidence. Soil solarization by covering it with a transparent polythene sheet for 6–8 weeks during summer effectively reduces the pathogen population (Katan 1980). Crop rotation and delayed planting are also very effective in controlling the disease. Use of healthy seed produced in a disease-free area helps in reducing seed-borne inoculum. Planting resistant cultivars is the most efficient measure in controlling the disease. A number of resistant varieties are available. It is important to know which race is present in the soil and selection of varieties that are resistant to the targeted race is crucial. Seed-borne inoculum can be controlled by treating the seed with fungicides such as Benlate-T and Bavistin (Haware et al. 1978). Some biocontrol agents may also be effective but additional research is needed for practical applications (Bhan and Chand 1998).

18.3.4 *Alternaria* Blight

18.3.4.1 Distribution and Economic Importance

Alternaria blight is a seed- and soil-borne disease considered serious in parts of Bangladesh and India (Nene et al. 2012).

18.3.4.2 Symptomatology

Like *Ascochyta* blight and *Botrytis* grey mould, *Alternaria* blight also appears at flowering and podding stage when the crop has built up its maximum canopy. Weather conditions (temperature and humidity) that favour the development of this disease are similar to those for *Ascochyta* blight and *Botrytis* grey mould, and all three diseases can occur together (Nene et al. 2012). Shedding of lower leaves and sparse podding are the most obvious symptoms of the disease. Infection is generally severe on leaves. Initially, lesions on leaflets are water-soaked, small, circular and purple (Fig. 18.4). These lesions

Fig. 18.4 *Alternaria* blight of chickpea

are surrounded by chlorotic tissues without definite margins (Fig. 18.4). Lesions later turn brown to dark brown, under high humidity, they coalesce and cover tenfold area. On the stems, the lesions are elongated and are brown to black in colour. The infected flowers die (Fig. 18.4). On the pods, the lesions are circular, slightly sunken and irregularly scattered. Affected pods turn dirty black. On mature pods, the lesions remain as localized, tiny and black superficial flecks. Infected seeds are shrivelled. Under favourable weather conditions, the entire foliage can die (Nene et al. 2012).

18.3.4.3 Aetiology

This disease is caused by *Alternaria alternata* (Fr.:Fr.) Kiessler. The extent of seed-borne infection by *Alternaria* blight disease was studied on chickpea seeds collected from infected pods using the standard blotter method. The cultural and morphological characters of the fungus observed from infected pods were characteristic of *Alternaria alternata*, which was recovered from all the surface unsterilized seeds indicating that the pathogen is seed-borne (Gurha et al. 2002).

18.3.5 *Colletotrichum* Blight

18.3.5.1 Distribution and Economic Importance

Colletotrichum blight is a minor seed- and soil-borne disease reported only from India (Nene et al. 2012).

18.3.5.2 Symptomatology

The disease can kill the plants at any stage of crop growth, depending on the weather conditions and amount of inoculum present (Fig. 18.5). Plants and branches that have dried up and get scattered throughout the field are an indication of *Colletotrichum* blight (Fig. 18.5). On seedlings, two kinds of symptoms can be observed: elongated, sunken, dark brown spots on the lower part of the stem, extending to the root, and wilting and drying due to severe collar and root infection (Fig. 18.5). In adult plants, lesions are seen on all the above-ground parts. On leaves and pods, lesions are

Fig. 18.5 *Colletotrichum* blight of chickpea

circular to elongate, sunken at the centre and with yellow margins. On stems they are elongated and black (Nene et al. 2012).

18.3.5.3 Aetiology

The disease is caused by *Colletotrichum dematium* (Pers. ex Fr.) Grove. The disease is generally severe when the crop is sown early (September) when the temperatures are high (25–30 °C) and the young crop is caught in the rains during late September or early October. The disease does not normally occur in the post rainy season crop, but if there are unusual rains, the disease can affect the crop. The fruiting bodies (acervuli) are scattered within the affected tissues. The fungus penetrates the pod wall and infects the seed (Nene et al. 2012).

18.4 Pigeonpea (*Cajanus cajan* (L) Millsp)

It is an important grain legume crop of rainfed agriculture in the semiarid tropics. The Indian subcontinent, eastern Africa and Central America are the world's three main pigeonpea-producing regions. Pigeonpea is cultivated in more than 25 tropical and subtropical countries, either as a sole crop or intermixed with such cereals as sorghum (*Sorghum bicolor* (L.) Moench), pearl millet (*Pennisetum glaucum* (L.) R. Br.) or maize (*Zea mays* L.) or with legumes, e.g. groundnut (*Arachis hypogaea* L.). Being a legume, pigeonpea enriches the soil through symbiotic nitrogen fixation. A short-day plant with a deep root system, pigeonpea tolerates drought, but is highly sensitive to waterlogging. The crop has many uses; fresh pigeonpeas are eaten as a vegetable, the grain is cooked and eaten as *dhal* (dry split cotyledons), the wood is used as fuel and the leaves and husks provide livestock feed. Diseases are major biological constraints to production, and more than 60 pathogens including fungi, bacteria, viruses, mycoplasma and nematodes can infect pigeonpeas. Fortunately, only a few of them cause economic losses. Of these, sterility mosaic and witches' broom are region-specific, whereas others such as *Fusarium* wilt are widespread across regions (Reddy et al. 1993).

18.4.1 *Fusarium* Wilt (*Fusarium udum,* Butler)

18.4.1.1 Distribution and Economic Importance

The disease was reported first by Butler (1906) who described the causal fungus as *Fusarium udum* Butler. Snyder and Hansen (1940) renamed the fungus as *Fusarium oxysporum* f. sp. *udum* (Butler) Snyd. and Hans. It is reported from the Bangladesh, Ghana, Grenada, India, Indonesia, Kenya, Malawi, Mauritius, Myanmar (Burma), Nepal, Nevis, Tanzania, Thailand, Trinidad and Tobago, Uganda and Venezuela. The annual losses due to wilt have been estimated at US$71 million in India and US$5 million in eastern Africa. It is the most serious disease of pigeonpea in India and is especially destructive in parts of Maharashtra, Madhya Pradesh, Uttar Pradesh and Bihar. Continuous cropping of pigeon pea in the same field may lead to as much

as 50% or more plant mortality due to wilt. In Bihar and Uttar Pradesh, 5–10% damage of standing crop is a common feature every year (Singh 1973). The infected plants show signs of gradual chlorosis and wilting about 5–6 weeks after sowing. In the field, the disease generally appears in large patches and in severe cases more than 50% plants in the field may be killed (Sen Gupta 1974). An International survey including Asia, Africa and America, conducted by ICRISAT from 1975 to 1980, annual losses in grain yield due to wilt were reported to be up to US$ 36 million (Kannaiyan et al. 1984). In eastern Africa, losses were estimated at US$ 5 million (Kannaiyan et al. 1984). Losses caused by the disease are dependent on the stage of wilt occurrence. If wilt occurs prior to podding, loss is total; however, only partial loss may result if wilt occurs at pod filling stage or later (Kannaiyan and Nene 1981). If wilt occurs during pod filling, the seed may become infected. Such seed may become a source of primary inoculum if not properly treated with fungicides. The total loss due to wilt disease is approximately 97,000 ton per year in India (Saxena et al. 2002).

18.4.1.2 Symptomatology

The disease is seed- and soil-borne. The fungus can survive on infected plant debris in the soil for about 3 years. Wilt symptoms usually appear when plants are at flowering and podding stages but sometimes occur earlier when plants are 1–2 months old. Patches of dead plants in the field when the crop is flowering or podding are the first indication of wilt (Fig. 18.6). The most characteristic symptom is a purple band extending upwards from the base of the main stem. This band is more easily seen in pigeonpeas with green stems than in those with coloured stems. Partial wilting of the plant is a definite indication of *Fusarium* wilt and distinguishes this disease from termite damage, drought and *Phytophthora* blight that kills the whole plant (Fig. 18.6). Partial wilting is associated with lateral root infection, while total wilt is due to tap root infection. The other characteristic symptom of wilt is browning of the stem tissue in the region of the purple band and browning or blackening of the xylem which is visible when the main stem or primary branches are split open (Fig. 18.6). The intensity of browning or blackening decreases from the base to the tip of the plant. Sometimes, branches (especially lower ones) dry, even if there is no band on the main stem (Fig. 18.6). These branches have die-back symptoms with a purple band extending from tip downwards, and intensive internal xylem blackening. When young plants (1–2 months old) die from wilt, they may not show the purple band symptom but have obvious internal browning and blackening. Plants infected by *F. udum* also exhibit a series of leaf symptoms before they die, including loss of leaf turgidity, interveinal clearing and chlorosis to bright yellow (Reddy et al. 1993).

18.4.1.3 Aetiology

The fungus mycelium is hyaline and produces three types of spores within the host tissue as well as in cultures, namely, microconidia, macroconidia and chlamydospores. Formation of these conidia depends on nutrition and other factors. Microconidia are small, elliptical or curved, unicellular or 1–2 septate, and measure 5–15 × 2–4 μm.

Fig. 18.6 *Fusarium* wilt of pigeonpea

They are formed free on hyphal branches. In cultures, a dozen or more of them may be held together in a ball or false head. Macroconidia are produced in small cushions of stromatic mycelium on the surface of the host near ground level. The stromatic bases (sporodochia) are tubercular in culture media. The macroconidia are long, curved (fusaroid), with prominent epical hook, and notched at the base, septate (3–4 septa). The presence of the prominent epical hook distinguishes the species from *Fusarium oxysporum*. Chlamydospores are also formed in the host as well as in old cultures. They develop from any cell of the hypha, often from cells of the macroconidium. The cells round off and become thick walled to form chlamydospores. These spores are oval to spherical, single or in chains, terminal or intercalary and persist in the soil for long. Rai and Upadhayay (1982) have reported *Gibbrella indica* Rai and Upadhyay as the perfect stage of *F. udum*. The perfect stage *Gibberella indica* is usually found on exposed roots and collar region of the stem up to the height of 35 cm above the ground level. The mature perithecia are superficial, commonly aggregated, and subglobose to globose, sessile, smooth walled and dark violet. Asci are eight spored, mostly subcylindrical, and broader in the middle, with a short stalk, a narrow apex and a central apical pore. Ascospores are ellipsoidal to ovate, hyaline, commonly two celled rarely three to four celled and constricted at the septa. In culture these spores germinate to produce short or long conidiophores bearing micro- and macroconidia which are pathogenic to pigeonpea. The fungus is heterothallic and single ascospore cultures do not produce perithecia. When culture

from different strains are grown together, perithecia are formed after 25 days at 18–22°C. The ascospores germinate to produce micro- and macroconidia.

18.4.1.4 Management

Bose (1939) recommended rotation of pigeonpea with tobacco as a possible means of control through the adverse effect of the root exudates of tobacco on the pathogen. Work conducted at the Indian Agricultural Research Institute, New Delhi, has shown that mixed cropping with sorghum also reduces the disease incidence. Vasudeva and Srinivasan (1952) and Vasudeva et al. (1962, 1963) demonstrated that a strain of *Bacillus subtilis* produces an antibiotic (bulbiformin) that inhibits the growth of *F. oxysporum* f. sp. *udum* in sterilized soil. However, the bacterium could not be utilized on a field scale as the antibiotic secreted by the bacterium is unstable in unsterilized soil. Kaiser and Sen Gupta (1969) demonstrated a significant reduction in the incidence of pigeonpea wilt as a result of preinoculation of the pigeonpea seedlings with some nonpathogenic strains of *F. oxysporum*. However, this cross protection has not yet been tested under field conditions. The best method of control is the use of resistant varieties.

18.5 Cowpea (*Vigna unguiculata* (L.) Walp)

It is widely grown for the consumption of its leaves, green pods and grain. It is the most important legume, providing a major source of the protein intake of rural and poor urban people of Africa and other parts of the world. The major constraints to increase on-farm production and to widespread cultivation of cowpea are the severe effects of a large number of pests and diseases.

18.5.1 Anthracnose

18.5.1.1 Distribution and Economic Importance

The disease is widely distributed, being present in almost all areas where beans (*Phaseolus vulgaris*) are grown. Isolates from cowpea have been obtained from Nigeria and other parts of Africa, India and Brazil. The disease is particularly severe in monocropped cowpeas in which it can cause up to 50% loss in yield (Singh and Allen 1979).

18.5.1.2 Symptomatology

All above ground parts can be affected but anthracnose is chiefly a stem disease in cowpea. Individual lesions are lenticular to sunken and tan to brown in colour. Lesion size and distribution depend on varietal susceptibility. Highly susceptible lines develop large spreading lesions which rapidly merge to girdle stems, branches, peduncles and petioles (Mordue 1971; Onesirosan and Barker 1971; Williams 1975).

18.5.1.3 Aetiology

This disease is caused by *Colletotrichum lindemuthianum*. Anthracnose can be distinguished from scab by the presence of black setae, and from the related brown blotch fungi by the shape of the conidia. Primary inoculum may come from seed (40% seed transmission) or from diseased plant debris. Secondary spread is rapid during cool, wet weather. Pathogenic variants occur (Mordue 1971; Onesirosan and Barker 1971; Williams 1975).

18.5.2 *Fusarium* Wilt

18.5.2.1 Distribution and Economic Importance

Fairly widespread, being reported from North and South America, Asia and Australia. Reliably recorded in tropical Africa only from Nigeria and Uganda. Locally damaging. Hosts include cowpea and soybean (Singh and Allen 1979).

18.5.2.2 Symptomatology

Leaves of infected plants are limp and yellowed and in young plants a rapid wilt leads to death. Older plants are stunted, leaves turn yellow and then fall and the plant gradually wilts. The vascular tissue is typically necrotic, and it is this symptom, and the presence of characteristic spores which distinguish the disease from the stem rots. *Fusarium solani* causes a collar and root rot of cowpea in certain areas of tropical Africa and America, while *Verticilttum albo-atrum* causes a vascular wilt of cowpea in North America and Australia (Holliday 1970; Oyekan 1975).

18.5.2.3 Aetiology

Fusarium oxysporum f. sp. *tracheiphilum*. The pathogen is soil-borne and probably also seed transmitted. Three pathogenic races are recognized (Holliday 1970; Oyekan 1975).

18.5.3 *Cercospora* Leaf Spot

18.5.3.1 Distribution and Economic Importance

Both pathogens are widespread in warmer regions, occurring on various legumes. They can cause considerable leaf spotting of cowpea after flowering when defoliation can lead to yield losses of up to 20% (*C. canescens*) and over 40% (*C. cruenta*). *C. canescens* is the more important (Singh and Allen 1979).

18.5.3.2 Symptomatology

C. canescens produces circular to irregular cherry-red to reddish brown lesions, up to 10 mm diameter. *C. cruenta* spots begin as a chlorosis (yellowing) on the leaf upper surface which becomes dotted with spots of dead tissue that enlarge until the whole lesion area is necrotic (Fig. 18.7). On the lower leaf surface, *C. canescens*, lesions are red whereas the lower surfaces of the leaves infected by *C. cruenta* have

Fig. 18.7 *Cercospora* leaf spot of cowpea

areas of profuse sporulation in which the masses of conidiophores (structures bearing spores) appear as downy grey-black mats (Fig. 18.7) (Verma and Patel 1969; Mulder and Holliday 1975; Williams 1975; Schneider et al. 1976; Fery et al. 1977; Vakili 1977).

18.5.3.3 Aetiology

The disease is caused by *Cercospora canescens* and *C. cruenta*. Sources of primary infection are infected seed, alternate hosts and infected debris (Verma and Patel 1969; Mulder and Holliday 1975; Williams 1975; Schneider et al. 1976; Fery et al. 1977; Vakili 1977).

18.5.4 Target Spot

18.5.4.1 Distribution and Economic Importance

Very widely distributed on numerous host plant species; especially abundant in the tropics. Of minor importance to cowpea on which it develops late (Singh and Allen 1979).

18.5.4.2 Symptomatology

The lesions begin as dark reddish-brown circular spots, 1–2 mm in diameter, which expand with narrow concentric banding to become large target spots, 15 mm in diameter. Such leaf lesions are often associated with veinal necrosis. The fungus sometimes produces lesions on petioles and stems, but these remain small (1–3 mm in diameter) and do not show concentric banding. The early stages of target spot can be confused with *Cercospora canescens* infection, but the regular concentric banding of the lesions, conidia and conidiophore characteristics are diagnostic (Ellis and Holliday 1971; Williams 1975).

18.5.4.3 Aetiology

This disease is caused by *Corynespora cassiicola* (= *Cercospora vignicola*). The conidia are wind-dispersed. The fungus is seed-borne and survives on host debris for up to 2 years. Various fungicides effectively control the disease, and sources of resistance are known (Ellis and Holliday 1971; Williams 1975).

18.5.5 *Septoria* Leaf Spot

18.5.5.1 Distribution and Economic Importance

Though *S. vignicola* is recorded from eastern Africa and India it appears to be less widely distributed and less important than, *S. vignae*, at least in the savannah zones of tropical Africa where Septoria leaf spot can be damaging (Singh and Allen 1979).

18.5.5.2 Symptomatology

Lesions caused by *S. vignae* are dark red, circular to irregular, 2–4 mm in diameter and appearing similar on both leaf surfaces. Spots are often concentric ringed, and sometimes raised, giving the leaf a freckled appearance (Williams 1975).

18.5.5.3 Aetiology

The disease is caused by *Septoria vignae* and *S. vignicola*. Black fruiting bodies (pycnidia) on the lesions contain septate (several celled), thread-like conidia. Species of the related *Chaetoseptoria* occur on cowpea leaves in North and Central America, while *Aristastoma* spp., in which the fruiting bodies bear bristles, cause pinkish grey, spreading and freckled lesions on cowpea leaves in southern Nigeria. It is likely to be seed-borne. There is evidence of varietal differences in susceptibility in northern Nigeria (Williams 1975).

18.5.6 *Ascochyta* Blight

18.5.6.1 Distribution and Economic Importance

A major disease of cowpea (and many other legumes) under humid conditions at medium elevations in eastern Africa and in Central America. Often devastating, causing extensive defoliation (Singh and Allen 1979).

18.5.6.2 Symptomatology

Young leaf spots are irregularly circular with grey to brown centres surrounded by a yellow halo. Such lesions become zonate and, under favourable conditions, spread rapidly causing extensive blighting of leaves, pods and stems. The large, concentrically ringed lesions are characteristic of the disease (Angus 1962–66; Sutton and Waterson 1966; Moreno 1975).

18.5.6.3 Aetiology

The incitant of disease is *Ascochyta phaseolorum*. Dark pycnidia immersed in the host tissue; these contain two-celled ovoid conidia. The disease is seed-borne and probably survives on infected plant debris.

18.5.6.4 Management

The disease spreads more rapidly in monoculture than when intercropped with maize which may act as a barrier to disease spread. Though some cowpea varieties possess low levels of resistance, the use of clean seed and cultural practices such as rotation are recommended control measures (Angus 1962–66; Sutton and Waterson 1966; Moreno 1975).

18.5.7 *Colletotrichum* Brown Blotch

18.5.7.1 Distribution and Economic Importance

The pathogens are widely distributed in the tropics and subtropics. Brown blotch of cowpea is a newly recognized disease in Nigeria where it may cause significant losses especially from pod infection. Brown blotch has also been observed in Upper Volta and Zambia (Singh and Allen 1979).

18.5.7.2 Symptomatology

The symptoms of the disease are purplish brown discolouration of petioles, leaf veins, stems, peduncles and, especially, pods. Discolouration may be accompanied by cracking of stems. Pod infection leads to distortion and maldevelopment of pods which bear black fruiting bodies of the causal fungi. Symptoms first appear either at the stem base before flowering, or on pedicels (floral cushion) following flowering, the latter being characteristic. The disease is seed-borne, and the pathogens probably also survive on infected plant debris (Emechebe 1981).

18.5.7.3 Aetiology

The disease is caused by *Colletotrichum capsici* and *C. truncatum*. *C. lindemuthianum* also occurs on cowpea pods but the brown blotch fungi differ in that their conidia are boat-shaped (Emechebe 1981).

18.5.8 Scab

18.5.8.1 Distribution and Economic Importance

This disease is reported from East Africa and Central America and causes severe damage of cowpea in Surinam. A very similar disease, whose cause is presumed to *Elsinoe*, causes extensive damage to cowpea crops in northern Nigeria. *E. phaseoli* also causes scab of lima bean and bean (Singh and Allen 1979).

18.5.8.2 Symptomatology

Scab infections lead to development of silvery grey, circular to oval lesions on stems, petioles, peduncles and pods. In severe infections, such lesions coalesce, causing distortion. Leaves of diseased plants are often cupped and bear numerous small whitish scab lesions along the veins. The stem symptoms can be confused with anthracnose but are greyish not tan-brown, and are more often circular.

18.5.8.3 Aetiology

The disease is caused by *Elsinoe phaseoli*. The pathogen has been detected within the seed coat and on its surface; it survives on host debris and air dispersal is also suspected. *Cladosporium vignae* also causes scab of cowpea.

18.5.8.4 Management

Crop rotation, sanitation and seed treatment are the recommended control measures. Cowpea lines differ in scab susceptibility (Sivanesan and Holliday 1971).

18.5.9 Bacterial Blight (Canker)

18.5.9.1 Distribution and Economic Importance

An important disease of cowpea in tropical Africa, America and India. Seedling mortality resulting from seed-borne infection may be up to 60%. However, yield losses from field infection have not been estimated.

18.5.9.2 Symptomatology

The initial symptoms of bacterial blight are tiny water-soaked dots on leaves. These dots remain small and the surrounding tissue dies, developing a tan to orange colouration with a yellow halo. On heavily infected leaves, the dead spots merge so that large areas of leaf are affected (Fig. 18.8). The pathogen also infects the stem, causing cracking (stem canker), and causes water soaking of pods from where the pathogen enters the seed.

Fig. 18.8 Bacterial blight (canker) of cowpea

18.5.9.3 Aetiology

This disease is caused by a bacterium *Xanthomonas vignicola.* The disease spreads rapidly during heavy rainfall, and during overhead irrigation. The pathogen is seed-borne, and probably survives on diseased crop residues (Watkins 1943; Sherwin and Lefebvre 1951; Patel and Jindal 1970; Williams 1975).

18.5.10 Bacterial Pustule (Bacterial Spot)

18.5.10.1 Distribution and Economic Importance

It is a widespread disease of both wild and cultivated cowpea in Nigeria. The disease occurs in Tanzania and apparently also in Brazil. Bacterial pustule appears to be less well adapted to the drier savannah regions of West Africa than is bacterial blight.

18.5.10.2 Symptomatology

The symptoms begin as tiny dark water-soaked dots on the undersurface of leaves. On susceptible varieties, these dots enlarge to become circular spots (1–3 cm diameter) which, when young, appear as raised dark water-soaked pustules on the lower surface of the leaf and as dark brown necrotic spots on the upper surface. Older, larger pustules become dry and sunken in the centre and water-soaked around the margin. Heavily infected leaves turn yellow and fall. Bacterial pustule is sometimes confused with pink rust but careful examination reveals the conical, not greasy, pustules of the latter.

18.5.10.3 Aetiology

This disease is caused by a bacterium *Xanthomonas* sp. The disease spreads rapidly during rainy weather and by overhead irrigation. The pathogen is seed-borne. Resistant varieties are available but there is evidence that some sources of resistance are race-specific (Williams 1975; Patel 1978).

18.5.11 *Cowpea (Severe) Mosaic Virus* (CSMV)

18.5.11.1 Distribution and Economic Importance

It is widespread in tropical and subtropical America. In Brazil, yield losses of 60–80% are caused by CSMV.

18.5.11.2 Symptomatology and Aetiology

A range of mosaics: The disease is sap and seed-borne (10%) and transmitted by several beetles including Ceratoma spp. *Phaseolus lathyroides*, and other common weeds in tropical America may act as reservoirs of CSMV (Shepherd 1964; Alconero and Santiago 1973; Diaz 1974; Lima and Nelson 1977).

18.5.12 *Cowpea (Yellow) Mosaic Virus* (CYMV)

18.5.12.1 Distribution and Economic Importance

The disease is known from East (Kenya, Tanzania) and West (Nigeria, Togo) Africa; essentially an African virus though occasionally reported from America (Surinam, U.S.A.). CYMV causes yield losses of 80–100%; the earlier the infection, greater is the yield loss.

18.5.12.2 Symptomatology and Aetiology

Different virus isolates and different cowpea cultivars show different symptoms with systemic reactions ranging from none, or and inconspicuous green mottle, to severe mosaic, leaf distortion and blistering, and death of the plant (Fig. 18.9). CYMV is readily sap transmitted and is seed-borne at a low level (1–5%), but little initial seed-borne infection rapidly spreads through entire crops through the activity of the chief vector, *Ootheca mutabilis*. Other beetles, grasshoppers and thrips are also reported to be vectors (Wilson and Jose 1968; Bock 1971; Whitney and Gilmer 1974).

18.5.13 *Cowpea Aphid-Borne Mosaic Virus* (CAMV)

18.5.13.1 Distribution and Economic Importance

It is the most wide-spread cowpea virus, reported from U.S.A., Europe, Africa, the south-west Pacific and Australia. Within Africa, CAMV is known from Kenya, Tanzania, Uganda, Zambia, Nigeria, Morocco and Egypt. Yield losses of 13–87% are reported from Iran.

Fig. 18.9 *Cowpea (yellow) mosaic virus* (CYMV)

18.5.13.2 Symptoms

Various mosaics and mottling symptoms. Some strains of CAMV produce characteristic green-vein banding, but this is not sufficient for accurate diagnosis. The disease is sap, seed (0–40%) and aphid-transmitted (Bock 1973; Kaiser and Mossahebi 1975; Ladipo and Allen 1979).

18.6 Moongbean (Green Gram) (*Vigna radiata* (L.) Wilczek) and Urd Bean/Black Gram (*Vigna mungo* (L.) Hepper)

Greengram (*Vigna radiata* (L.) Wilczek) and Blackgram (*Vigna mungo* (L.) Hepper) are among the major legumes crops grown in warm season in India and Southeast Asia (Pandey et al. 2009). They are important pulse crops in our country after chickpea and pigeonpea. Both are short duration grain legume crops with wide adaptability, have low input requirement and have the ability to improve soil fertility by fixing atmospheric nitrogen. Both the crops are thought to be of Indian origin as evidenced by occurrence at archaeological sites in the Indian subcontinent (Sharma et al. 2011). The production of these two major pulses is adversely affected by several biotic stresses. Among biotic stresses, fungal foliar diseases especially *Cercospora* leaf spot (*Cercospora canescens* Ellis and Martin), web blight (*Rhizoctonia solani* Kuhn) and powdery mildew (*Erysiphe polygoni*) have emerged as the most important diseases (Akhtar et al. 2014).

18.6.1 Common Blight

18.6.1.1 Distribution and Economic Importance

Common blight is a serious disease in many of the important snap and dry bean-producing regions of the world. Although its effect on yield is difficult to estimate, workers have reported losses to be in the range of 10–45% (Hagedorn and lnglis 1986). Common bacterial blight spreads easily from infected seed and is the number one foliar disease of dry bean in Canada (Bailey et al. 2003).

18.6.1.2 Symptomatology

Symptoms first appear as water-soaked spots on the leaves and develop into necrotic lesions surrounded by a chlorotic border (Hall 1991). The disease is characterized by many brown, dry and raised spots on the leaf surface. When the disease is severe several such spots coalesce, the leaves become yellow and fall off prematurely. The lower surface of the leaf appears red in colour due to the formation of raised spots. The stem and pods also get infected. Symptoms on the leaves first appear as watersoaked spots. The spots enlarge initially into flaccid but then brown and necrotic lesions with lemon-yellow margins. Lesions often coalesce to cause extensive tissue damage leading to defoliation. Wilting is evident if the pathogen invades the plant vascular system. Under high humidity and high temperature, infection move to the pods. Pod lesions initially appear water soaked that

enlarge and become slightly sunken with a brick-red border (Schwartz et al. 2005). Yellow exudate may form on lesions (Schuster and Coyne 1977). Severe pod lesions can infect the developing seed and cause shrivelling and a butter-yellow discolouration (Swings and Civerolo 1993). Infected seed is the primary inoculum source for CBB and the use of disease-free seed is an important management strategy to control its spread (Bailey et al. 2003; Schwartz et al. 2005). Seed production areas with frequent rainfall and high humidity typically result in infected seed lots (Gillard et al. 2009).

18.6.1.3 Aetiology

The causal agent of common bacterial blight of bean *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye was first described in 1897 and has undergone a number of taxonomic revisions over the years (Bradbury 1986). It remains one of the most serious deterrents to bean production throughout the world. It is most serious in areas with warm and wet or humid conditions during the growing season. However, the minimal requirements for field infection, particularly temperature and humidity, have not been fully established (Saettler 1989), the disease occurs on all continents (Bradbury 1986). Its wide distribution is presumed to be due to efficient seed transmission. Economic losses due to CB result from reduced marketability of the seed, or even loss of seed production (Vidaver 1993). The bacteria is commonly seed-borne. As the seed germinates, bacteria contaminate the surface of the expanding cotyledon and spread to the leaves via natural openings and wounds, and eventually even to the vascular system. Throughout the season, bacteria can spread to other plant parts by wind-driven rains or hail, insects, farm implements or human activity. Localized lesions on pods and systemic invasion of pods lead to external and internal seed contamination. *Xanthomonas* can overwinter in seed and infested bean straw and can survive in seed for over 15 years. This is favoured by warm temperatures (28 °C) and high humidity (Zaumeyer 1930; Coyne and Schuster 1974a, b; Schuster and Coyne 1977).

18.6.2 Leaf Crinkle Disease

18.6.2.1 Distribution and Economic Importance

Urdbean leaf crinkle disease (ULCD) is an economically significant widespread and devastating disease resulting in extreme crinkling, puckering and rugosity of leaves inflicting heavy yield losses annually in major Urdbean-producing countries of the world. Urdbean (*Vigna mungo* L. Hepper) is relatively more susceptible than other pulses to leaf crinkle disease. Aphids, insects and whiteflies have been reported as vectors of the disease. The virus is also transmitted through sap inoculation, grafting and seed. The loss in seed yield in ULCD-affected urdbean crop ranges from 35 to 81%, which is dependent upon type of genotype, location and infection time (Gautam et al. 2016). The leaf crinkle of urd was first observed in Delhi and UP in 1966 (Williams et al. 1968). It has the potential of causing heavy losses. Almost all varieties of urd have been found susceptible to this disease. The average loss in yield could be 3–95%

depending upon the stage of plant growth at which infection occurs. Earlier the infection, greater is the loss, mainly due to reduction in pod number (Kadian 1982).

18.6.2.2 Symptomatology

The earliest symptoms appear on youngest leaves as chlorosis around some lateral veins and its branches near the margin. The leaves show curling of margin downwards. Some of the leaves show twisting. The veins show reddish brown discolouration on the undersurface which also extends to the petiole. Plants showing symptoms within 5 weeks after sowing, invariably remain stunted and majority of these die due to top necrosis within a week or two. Plants infected in late stages of growth do not show severe curling and twisting of the leaves but show conspicuous venial chlorosis any where on the leaf lamina. The disease develops in the fields mainly through seed or rubbing of diseased leaves with the healthy ones (Gautam et al. 2016). The characteristic symptom is the enlargement of the leaflets of affected trifoliate leaves followed by crinkling. These leaves then become leathery and thicker. The flower stalks produced from the axil of the affected leaves bear excessive number of small-sized flower buds at its tip. The sepals of these flower buds become thick and greener than the normal. Such buds very often break at their tips prematurely and possess a considerable sterility. This results in poor fruit set. The affected plants do not die till the harvest of the crop (Kolte and Nene 1970; Nene 1972).

18.6.2.3 Aetiology

This disease is caused by *Urdbean leaf crinkle virus* (ULCV). The virus is seed-borne and found to be transmitted through seeds to the extent of 18–39% in variety 'T-9' (Kolte and Nene 1972). The virus is confined to cotyledons and embryo (Sharma and Dubey 1981). The virus appears to be spherical with a diameter of 25–30 nm (Bhaktavatsalam et al. 1983).

18.6.3 *Cercospora* Leaf Spot

18.6.3.1 Distribution and Economic Importance

Cercospora leaf spot was first known to occur in Delhi, India (Munjal et al. 1960) and is prevalent in all parts of the humid tropical areas of India, Bangladesh, Indonesia, Malaysia, Philippines, Taiwan as well as Thailand (Pandey et al. 2009). It becomes severe in the wet season causing up to 100% yield loss (Quebral and Cagampang 1970; Amin and Singh 1987; Grewal 1988; Iqbal et al. 1995; Pandey et al. 2009).

18.6.3.2 Symptomatology

It usually occurs in a severe form, causing heavy yield losses. Numerous small spots with pale brown centre and reddish brown margin are produced on leaves (Fig. 18.10). Similar spots also occur on branches and pods. Under favourable environmental conditions, severe leaf spotting and defoliation occur at the time of

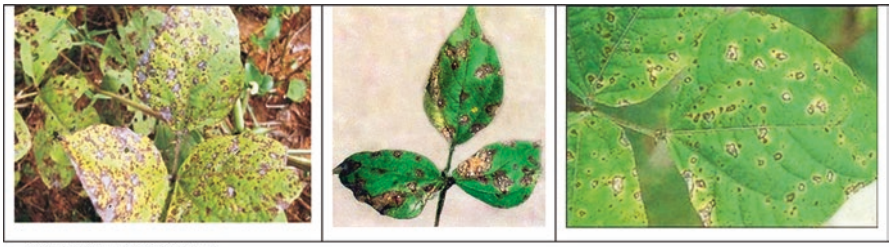

Fig. 18.10 *Cercospora* leaf spot of moong bean

flowering and pod formation (Iqbal et al. 2004, Dubey and Singh 2010). The disease starts appearing about 30–40 days after sowing.

18.6.3.3 Aetiology

The incitant of the disease is *Cercospora canescens*. The pathogen can be seed-borne. Depending upon the temperature and humidity, it spreads rapidly in susceptible varieties causing premature defoliation and reduction in size of pods and grains (Grewal et al. 1980; Akhtar et al. 2014).

18.6.4 Root Rot and Leaf Blight

18.6.4.1 Distribution and Economic Importance

This disease occurs in many U.S. states, Brazil and the Philippines. Losses of 5–10% are common, but 60% yield losses have been reported in Brazil (Hagedorn and lnglis 1986).

18.6.4.2 Symptomatology

Reddish-brown, elongated, cankerous lesions are present on root and lower hypocotyl. Minute brown sclerotia may develop on cankers. Diseased young plants show red colouration to the pith and may die. Lesions or girdling of an older stem may cause death. If weather is favourable, fungus strands bind above-ground parts into a mouldy mat. The pathogen is the soil-borne fungus.

18.6.4.3 Aetiology

The disease is caused by a fungus *Thanatephorus cucumeris* (*Rhizoctonia solani*). This disease spreads through infested soil. It can be seed-borne. Optimum soil temperature is 18 °C. Normal moisture regimes are adequate, but increased soil moisture often results in more severe disease (Christou 1962; Weinhold et al. 1969; Baker and Martinson 1970; Leach and Garber 1970).

18.6.5 Anthracnose

18.6.5.1 Distribution and Economic Importance

Anthracnose occurs in many areas of the world and is of major importance on susceptible dry and snap bean cultivars. In the United States, anthracnose was once serious in eastern and central states, but now the use of disease-free seed has virtually eliminated yield losses (Hagedorn and lnglis 1986).

18.6.5.2 Symptomatology

The fungus attacks all aerial parts and at any stage of plant growth. Symptoms appear as circular, black, sunken spots with dark centre and bright red orange margins on leaves and pods (Fig. 18.11). In severe infections, the affected parts wither off. Seedlings get blighted due to infection soon after seed germination. Symptoms on above-ground parts of the plant appear as brick-red to dark brown lesions. On stems, leaf petioles and veins on the lower surface of leaves, lesions are usually sunken and elongated. On pods, lesions are sunken and circular. Infected seeds are usually discoloured and may have sunken lesions. During periods of moist weather, gelatinous masses of pinkish spores may develop in infected areas. The yield and vigour of the severely affected plants are greatly reduced.

18.6.5.3 Aetiology

The disease is caused by *Colletotrichum lindemuthianum.* The fungus survives from season to season on infected plant debris and in seed. When infected seed germinates, lesions develop on the cotyledons. Spores from lesions can be splashed by

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Fig. 18.11 Anthracnose of moongbean

rain and irrigation water, or spread by insects, animals, humans or cultivators. Anthracnose is favoured by cool temperatures 16 °C and wet conditions (Leach 1923; Goth and Zaumeyer 1965; Copeland et al. 1975).

18.6.6 Halo Blight Disease

18.6.6.1 Distribution and Economic Importance

Worldwide in occurrence, halo blight has repeatedly caused important economic losses (Hagedorn and lnglis 1986). Losses of up to 43% due to infection of green bean by *P. s.* pv. *phaseolicola* have been reported overseas (Allen et al. 1998). Both the tan spot and halo blight pathogens are seed-borne (Wood and Easdown 1990; Conde and Diatloff 1991; Saettler 2005a, b), and their presence in planting seed can pose a considerable threat. Often, very low levels of seed-borne infection can result in serious disease outbreaks under conducive conditions; Walker and Patel (1964) estimated that only two *P. s.* pv. *phaseolicola* infected seeds in 10,000 was needed to initiate an epidemic of halo blight on french bean if the weather conditions were conducive.

18.6.6.2 Symptomatology

On the leaves, small, angular, water-soaked spots appear first on the lower leaf surface (Fig. 18.12). As these spots increase in size a characteristic halo of yellow tissue develops around each water-soaked spot (Fig. 18.12). The spots are 3–6 mm in diameter. The halo may be up to 2.5 cm in diameter. On pods, the oval water-soaked spots may increase up to 9 mm in diameter and become slightly sunken and reddish brown with age. A cream-coloured bacterial exudate is often found in pod lesions. Both leaf and pod lesions often coalesce. The upper foliage of diseased plants develops a characteristic yellow colour. Infected seed may be smaller than normal, have a wrinkled seed coat and may be discoloured.

18.6.6.3 Aetiology

Pseudomonas syringae pv. *phaseolicola.* The bacterium is a seed-borne pathogen. Several races are known. The pathogen can exist in infected plant tissues for up to 1 year and spreads by splattering water or wind-blown rain. Disease development is

Fig. 18.12 Halo blight of moong bean

favoured by humid, cloudy conditions. Halo expression is favoured by cool temperatures of 16–20 °C rather than warmer weather, but the causal organism spreads and disease develops very well in warm temperatures (Walker and Patel 1964; Patel and Walker 1966; Grogan and Kimble 1967; Wharton 1967; Hagedorn et al. 1974; Taylor and Dudley 1977; Ryley et al. 2010).

18.6.7 *Alternaria* Leaf Spot

18.6.7.1 Distribution and Economic Importance

Alternaria leaf spot is occasionally destructive in the United States (central Wisconsin, and central and western New York), Latin America and the United Kingdom (Hagedorn and lnglis 1986).

18.6.7.2 Symptomatology

On the leaves, small, brown irregular-shaped lesions develop into large, grey-brown oval lesions with concentric rings. Leaf lesions do not always cross over major leaf veins, in such cases lesions may be angular in shape. When several lesions coalesce, a large portion of the leaf area becomes necrotic. Sometimes the necrotic areas fall out and the leaf has a shot-hole appearance. Premature defoliation beginning with the lowest leaves may occur. Reddish-brown lesions that merge into long streaks develop on the pods from small water-soaked flecks. Old senescing leaves and pods are more susceptible than young leaves and pods.

18.6.7.3 Aetiology

The disease is caused by *Alternaria alternata* and other *Alternaria* species. The fungus produces spores on diseased plants and which are easily disseminated by wind, rain, insects and seed. Cool and wet weather in which leaves remain wet for periods of 24 hours or longer are essential for spore germination and infection (Saad and Hagedorn 1969; Abawi et al. 1977; Russell and Brown 1977).

18.6.8 Angular Leaf Spot

18.6.8.1 Distribution and Economic Importance

Angular leaf spot occurs worldwide but is primarily a disease of the tropics and subtropics. Reported yield losses range from 10 to 50% (Hagedorn and lnglis 1986).

18.6.8.2 Symptomatology

Diseased plants are characterized by angular spots on the leaves. Initially such lesions are tannish grey, but later they become dark brown or black. As they increase in size, several spots may coalesce and large proportions of the leaf area become infected and chlorotic. During periods of high humidity, the undersurface of the leaves may have a black felt-like appearance due to formation of spores by the

pathogen. Premature defoliation of the plant occurs. Diseased pods show circular spots with reddish-brown centres. Severely diseased plants have reduced vigor and poor yield.

18.6.8.3 Aetiology

Isariopsis griseola The fungus overwinters mostly in infected bean debris but sometimes in seed. The spread is mostly by wind-blown spores. Humid conditions favour disease development. The optimum temperature for symptom development is 24 °C, but it can develop over a wide range of temperatures, 16–28 °C (Cardona-Alvarez and Walker 1956; Santos-Filho et al. 1976; Singh and Sharma 1975).

18.6.9 *Ascochyta* Leaf and Pod Spot

18.6.9.1 Distribution and Economic Importance

Ascochyta leaf and pod spot of bean occur quite commonly in Latin America and occasionally in many other parts of the world but are somewhat rare in the United States. It may occasionally be of major economic importance but, on a world basis, it is of moderate importance (Hagedorn and lnglis 1986).

18.6.9.2 Symptomatology

The large, light to dark brown leaf lesions show a series of conspicuous concentric rings, giving the lesion a zonate appearance. A lesion may cover 1/4 to 1/2 of the leaf surface. Small, black pycnidia are formed in diseased tissue. Pod lesions are dark brown, slightly sunken and zonate. They also commonly have pycnidia. Severe, multiple infections result in premature defoliation and reduced plant vigour. Stem girdling can cause plant collapse.

18.6.9.3 Aetiology

Ascochyta boltshauseri Sacc. and *A. phaseolorum* Sacc. These pathogens can be seed-borne and can be spread long distances. Local spread is accomplished by wind and rain-borne pycnidiospores. *Ascochyta* leaf and pod spot are favoured by cool-moderate temperatures of 16–24 °C and high humidity. Temperatures above 30 °C inactivate the pathogen. Rainy, overcast weather favours disease development (Sprague 1935, 1948).

18.6.10 *Bean Common Mosaic Virus* (BCMV)

18.6.10.1 Distribution and Economic Importance

Bean common mosaic occurs on snap and dry beans in nearly every country of the world. Yield losses may vary from 6% to 98% depending on the cultivar and time of infection (Hagedorn and lnglis 1986).

18.6.10.2 Symptomatology and Aetiology

Trifoliolate leaves usually show irregular-shaped, light-yellow and dark-green areas in a mosaic-like pattern. Leaves may have considerable puckering, stunting, malformation and/or downward curling. Sometimes infected leaves are narrower and longer than normal ones. Early infected bean plants are usually yellowish and dwarfed. Some bean cultivars display systemic vein necrosis in leaves, stems, roots or pods or localized necrotic leaf lesions; this condition is known as 'black root'. Infected pods may be chlorotic, shortened and possess a glossy sheen. Infected seed appears normal. It is sometimes called bean virus 1 or Marmor phaseoli, overwinters in weed hosts and in infected seed. Long distance spread is by infected seed. Locally, the virus may be transmitted from plant to plant mechanically, in pollen, or most commonly by aphids. Mosiac symptoms are best expressed at moderate temperatures of 20–25 °C and systemic vein necrosis at higher temperatures of 26–32 °C (Zaumeyer 1969; Alconero and Meiners 1974; Drijfhout 1978).

18.6.11 Other Virus Diseases

18.6.11.1 Yellow Mosaic Disease

Mungbean yellow mosaic India virus (MYMIV) belongs to Begomovirus genus. The disease caused is known as Yellow mosaic disease (YMD) of legumes. The symptoms appear as small yellow streaks, developing into yellow mosaic. Infected plants produce fewer pods and the number of seeds per pod is dramatically reduced (Varma et al. 1992). Typical yellow mosaic disease was first observed in the late 1940s in western India in Lima bean (*Phaseoius iunatus*), and in the 1950s in mungbean (*Vigna radiata*) in northern India (Nariani 1960). Since then YMD has spread throughout India causing enormous losses in the production of French bean, blackgram, cluster bean, groundnut, horsegram, hyacinth bean, moth bean, mungbean, Lima bean, pigeonpea and soybean. Based on sequence identities, two distinct Begomovirus species, *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV), causing YMD in grain legumes have been recognized (Fauquet et al. 2003). The YMD epidemic in northern India is caused by the variants of MYMIV, whereas in southern India variants of MYMV are more prevalent (Varma and Malathi 2003). The annual yield losses in India due to YMDs in three important leguminous crops (blackgram, mungbean and soybean) are estimated to be about US$300 million (Varma et al. 1992).

18.6.11.2 Mosaic Mottle

The mosaic mottle of urdbean, as it is called now, was reported first by Shahare and Raychaudhuri (1963) under the name mosaic disease of urd. Later Nene and Srivastava (1972) also observed this disease on urd bean and described it under the name mosaic mottle. Singh and Nene (1978) identified the causal virus of urdbean mosaic mottle as a strain of *Bean Common Mosaic Virus*. The symptoms of mosaic mottle in urdbean appear first in the primary leaves in the form of slight mottling

with reduced leaf size and downward rolling of the margins. On subsequent leaves, the symptoms appear in the form of irregular light green patches alternating with normal green areas. The margins show upward rolling and the leaf size is considerably reduced. Later these leaves show light puckering and blistering. Such leaves become rough and brittle with age. The infected plants show reduction in overall growth. The inflorescence is either partially or wholly changed into leaf-like structure depending upon the age of plant. Seedlings developing from infected seeds may show symptoms at either primary leaf stage or on the first or succeeding second trifoliate leaves (Nene and Srivastava 1972; Agarwal et al. 1977). The virus is reported to be internally seed-borne in embryo of urdbean seeds (Agarwal et al. 1979).

18.7 Lentil/Masoor (*Lens culinaris* Medikus)

Lentil plants are affected by a wide range of pathogens with fungal disease being the most important. These decrease productivity through infection and damage to leaves, stems, roots and pods and reduce marketability by discolouring seed. Most major economically important diseases are found in all lentil-growing regions of the world, e.g. *Ascochyta* blight, *Fusarium* wilt and anthracnose. Certain virulent pathotypes of a pathogen have restricted geographical range (e.g. CtO pathotype of anthracnose in Canada). The major fungal diseases of lentil were described with descriptions of the causal organism, symptoms produced on the plant, epidemiology and disease management and control (Taylor et al. 2007).

18.7.1 *Ascochyta* Blight

18.7.1.1 Distribution and Economic Importance

It is one of the most important biotic constraints to lentil production. It attacks all above-ground plant parts at any growth stage under favourable conditions and causes reduction in yield and seed quality. The disease is prevalent throughout the world and has been reported to cause yield losses of up to 70%, 30–50% and 50% in Canada, the United States and Australia, respectively (Gossen and Morrall 1983; Kaiser 1992; Brouwer et al. 1995).

18.7.1.2 Symptomatology

The symptoms of the disease include lesions on leaves, petioles, stems and pods. The irregularly shaped lesions on leaves, petioles and stem are tan and darker brown on pods and seeds (Fig. 18.13). Black pycnidia are visible in the centre of mature/older lesions. In severe infection, lesions can girdle the stem, leading to breakage and subsequent death of all tissues above the lesion (Fig. 18.13). Heavily infected seeds are shrivelled and discoloured with whitish mycelium and pycnidia (Kaiser and Hannan 1986).

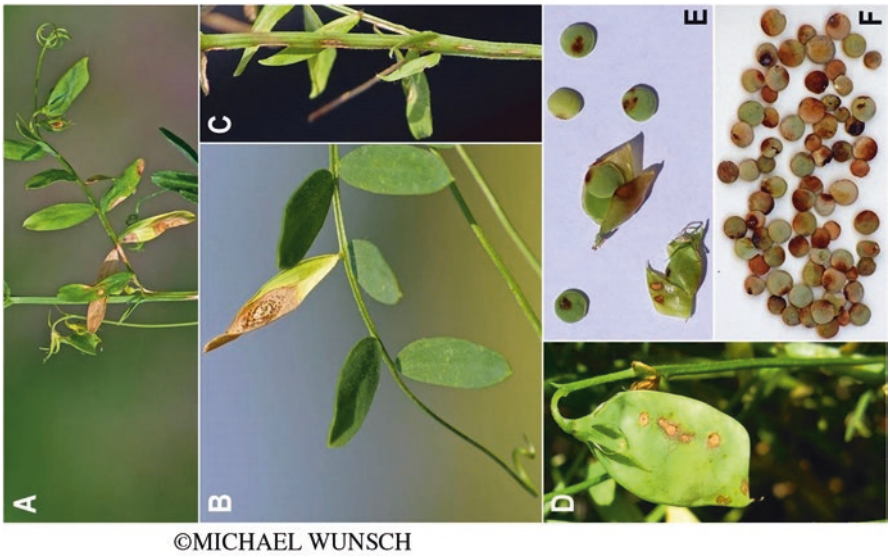

Fig. 18.13 *Ascochyta* blight of lentil

18.7.1.3 Aetiology

This disease is caused by *Ascochyta lentis* (Bond. and Vassil). There are two stages in the life-cycle of *A. lentis*, the asexual or anamorph stage and the sexual or teleomorph stage. The asexual stage is characterized by the production of pycnidia in the lesions on infected plants with a minute round ostiole (Bondartzeva-Monteverde and Vassilievsky 1940 cited by Agrawal and Prasad 1997). The pycnidia release conidia which are cylindrical, straight or rarely curved, round at the ends with a median septum. The teleomorph (*Didymella lentils*) was observed for the first time on over wintered lentil straw in 1992 in Idaho, USA (Kaiser and Hellier 1993), confirming the heterothallic nature of *A. lentis*, with two distinct mating types. Kaiser (1997) identified both mating types in isolates from Australia, Canada, Italy, Morocco, New Zealand, Pakistan, Spain, Syria, Turkey and the United States. Cool, wet weather is conducive to *A. lentis* infection, disease development and spread. The disease affects all the aerial parts of the plant and is seed-borne. Conidia may be dispersed under rain splash up to 15 cm (Pedersen et al. 1994), while wetness periods of 24–48 hours and temperatures of 10–15 °C are optimum for infection (Pedersen and Morrall 1994). The pathogen may also be dispersed by wind blown infected leaflets (Pedersen et al. 1994) and through infected seed (Kaiser and Hannan 1986). Kaiser and Hannan (1986) found *A. lentis* infection in seeds of 46 accessions from 30 countries. They also found the pathogen on seed from countries where the disease was previously unrecorded. Kaiser et al. (1989) stored infected lentil seed at 20, 5, −18 and −160 to −196 °C and proposed that the pathogen would survive in infected lentil seed as long as or longer than the seed remained viable.

18.7.1.4 Management

Losses due to *Ascochyta* blight can be minimized by crop rotation, early sowing to escape moist weather at harvest, the use of disease free seed and burning of diseased debris from the previous crop (Nene et al. 1988). A 3-year break between lentil crops reduced the amount of inoculum in the soil. Sun drying of lentil seed was found to be useful in controlling seed-borne inoculum (Beniwal et al. 1989). Hot water and dry heat treatment at 55 °C for 25 min and 70 °C for 24 h, respectively, inhibited fungal growth in the seed; however, seed germination declined drastically with the hot water treatment (Ahmed and Beniwal 1991). A large number of fungicides have been evaluated for control of seed-borne infection with benomyl, carbendazim, carbathiin, iprodion and thiobendazole reported to be effective in varying degrees (Morrall 1998; Bretag 1989). Metalaxyl and thiram have also been found to reduce fungal growth but thiram did not control the disease effectively in the field (Bretag 1989). In particular, Kaiser and Hannan (1987) reported greater seedling emergence from infected lentil seed and increased yield after treatment with thiobendazole and benomyl but thiobendazole showed a phytotoxic effect at 3 g or more a.i./kg of seed. The effects of foliar application of fungicides were studied by Beauchamp et al. (1986) who reported that captafol, chlorothalonil, folpet and metiram completely inhibited conidia germination. Seed yield increased and seed infection was reduced using single applications of these fungicides at early bloom to early pod set. Lentil breeding programs have developed resistant cultivars; however, knowledge of pathogenic diversity is important when choosing appropriate isolates to screen for resistance. Many studies have shown pathogenic diversity among isolates by assaying a set of host-specific differential genotypes or cultivars. However, since resistance to *A. lentis* has been found to be controlled by specific resistance genes (Ford et al. 1999; Nguyen et al. 2001), there, is likelihood that pathotypes of *A. lentis* have evolved that have qualitative differences on lentil genotypes.

18.7.2 *Fusarium* Wilt

18.7.2.1 Distribution and Economic Importance

Fusarium wilt of lentil is an important disease reported in every continent where lentil is grown except Australia (Beniwal et al. 1993; Tosi and Cappelli 2001). The disease may cause complete crop failure under favourable conditions and can be the major limiting factor for lentil cultivation in certain areas (Chaudhary and Amarjit 2002). The common name lentil wilt has been used to describe many general wilting and dying symptoms. Hence a number of pathogens have been reportedly associated with lentil wilt (Khare 1981), possibly because of the difficulty in species identification and confusion in the *Fusarium* taxonomy.

18.7.2.2 Symptomatology

Fusarium wilt usually occurs near or at reproductive stages (flowering to pod-filling) of crop growth. Symptoms include wilting of top leaves that resemble water deficiency, stunting of plants, shrinking and curling of leaves from the lower part of

the plants that progressively move up with the stems of the infected plant. Plants finally become completely yellow and die. Root symptoms include reduced growth with marked brown discolouration, tap root tips are damaged and proliferation of secondary roots above the area of tap root injury. Discolouration of vascular tissue in the lower stem may not always be visible. However, in India, the disease has also been reported to occur at the seedling stage. General symptoms at the seedling stage include seed rot and sudden drooping more like wilting and damping off (Khare 1980). Lower stems may be splitted to check for vascular discolouration. Although vascular discolouration is not always symptomatic of *Fusarium* wilt, the presence of discolouration would confirm the disease.

18.7.2.3 Aetiology

This disease is caused by *Fusarium oysporum* (Schlecht. Emend. Snyder & Hansen) f. sp. *lentis* (Vasudeva and Srinivasan). Like many other formae speciales of *F. oxysporun*, it has a very limited host range as it only infects lentil in nature (Khare 1980). In culture, the mycelium of the pathogen is hyaline, septate and profusely branched. Growth patterns on media vary from fluffy to appressed and vary in colour from no colour to pink. *F. oxysporum* f. sp. *lentis* produces three kinds of spores: microconidia; multiseptate macroconidia, which have a distinct foot cell and a pointed apical cell; and chlamydospores (Khare 1980). Microconidia are ovoid or kidney-shaped, hyaline and usually one celled. Macroconidia are long with pointed apical cell and notched basal cell and are two to seven celled. Chlamydospores are oval or spherical, one-celled and thick walled, formed singly in macroconidia or apical or intercalary in the hyphae. The disease is favoured by warm and dry conditions (Bayaa and Erskine 1998), with an optimal temperature of 22–25 °C. It is a soil-borne pathogen, although seed infestation and infection are common. The chlamydospores can survive in soil either in dormant form or saprophyticaIly for several years without a suitable host. A survey of soil samples from India found that *F. oxysporum* f. sp. *Ientis* was the most prevalent lentil pathogen (Chaudhary and Amarjit 2002). Synergistic interaction between *F. oxysporum* f. sp. *lentis* and root knot nematode *Meloidogyne jàvanica* was observed in lentil cultivars resistant or susceptible to *Fusarium* wilt. Presence of the nematode significantly increased wilt incidence, significant reduction in shoot length, root length and nodulation (De et al. 2001).

18.7.2.4 Management

The most economical management is through the use of resistant cultivars (Bayaa et al. 1997; Stoilova and Chavdarov 2006). Resistant or moderately resistant lentil cultivars significantly reduced wilt incidence and severity of root rot, and increased grain yield (Chaudhary and Amarjit 2002). Selecting cultivars that mature early and adjusting the planting date if possible can reduce disease incidence by escaping a portion of lentil growth from weather conditions favourable to the disease. The most suitable planting dates vary according to the different growing regions. Use of clean seed for sowing and/or the use of fungicidal seed treatments can eliminate or reduce contaminating inoculum sources. Since, the pathogen has a very restricted host range, a 3–5 year rotation will help to reduce inoculum level in field (Taylor et al. 2007).

18.7.3 *Botrytis* Grey Mould

18.7.3.1 Distribution and Economic Importance

Botrytis grey mould (BGM) of lentil is a serious but sporadic disease. Knights (1987) first reported the disease in Australia on lentil in the wet year of 1983 and since then the disease has caused considerable damage to commercial lentil crops grown throughout Victoria and South Australia (Lindbeck et al. 2003). In Canada, the disease was first reported in 1970 (Morrall et al. 1972) with serious epidemics of Botrytis stem and pod rot occurring from 1992 to 1994 (Morrall 1997). A series of cool, wet summers in those years provided ideal conditions for *Botrytis* epidemics to occur. The disease has been recorded on lentil in the United States in 1964 (Wilson and Brandsberg 1965) and in New Zealand in 1987 (Cromey et al. 1987). *Botrytis* grey mould has also been reported as being a serious problem throughout the subcontinent including Bangladesh (Gowda and Kaul 1982), Nepal (Karki 1993) and Pakistan (Bashir and Malik 1988; Iqbal et al. 1992). Brouwer et al. (2000) found only *Botrytis cinerea* to be a problem in lentil production in Pakistan but not the rest of the Indian subcontinent, despite *B. fabae* being a common pathogen in the region. On the South American continent, the disease has been reported as a production constraint in Colombia (Bascur 1993). *B. cinerea* has also been isolated from infected and dying plants in Chile (France et al. 1988) and from lentil with symptoms in the field in northern Egypt (Hamdi and Hassanein 1996). In Europe, the production of lentil is claimed to be limited by low profitability and its susceptibility to *Botrytis* in wet climates (Carrouee et al. 2000).

18.7.3.2 Symptomatology

All above-ground plant parts of lentil can be affected by *Botrytis* grey mould (Fig. 18.14). Depending on the location of the crop, symptoms may initially appear either on flowers and pods, or lower crop canopy (Fig. 18.14). The most damaging symptoms become apparent after the crop has reached canopy closure and a humid

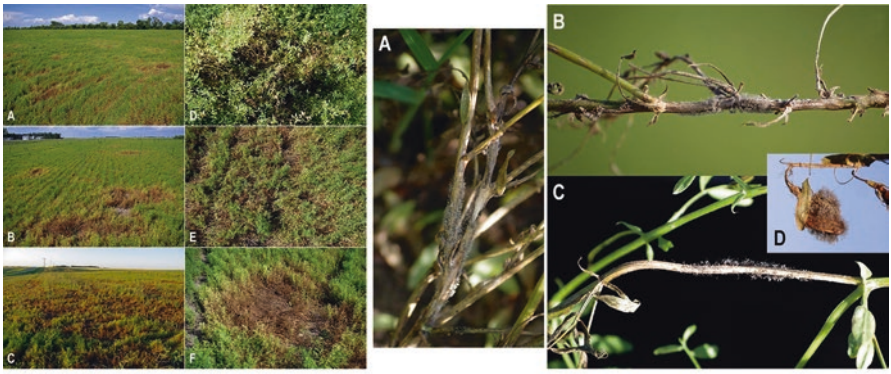

Fig. 18.14 *Botrytis* grey mould of lentil

microclimate is produced under the crop canopy. The disease first appears on the lower foliage as discrete lesions on leaves which are initially dark green, but turn greyish-brown, then creamy as they age, they enlarge and coalesce to infect whole leaflets. Severely infected leaves senescence and fall to the ground. These can often act as a secondary source of inoculum by lodging in leaf and stem axils and initiating stem infections. If the canopy remains humid for extended periods infection can spread to the lower stems which quickly become girdled and covered with a furry layer of conidiophores, eventually causing stem death and whole plant infection (Fig. 18.14). Death of plants can often occur before the onset of flowering and pod filling. Infection will continue to spread resulting in patches of dead plants within crops (Bayaa and Erskine 1998).

18.7.3.3 Aetiology

This disease is caused by *Botrytis fabae* (Sard) (teleomorph: *Botryotinia fabae*) and *B. cinerea* (Pers., Fr.) (teleomorph: *Botryotinia fuckeliana*). Colonies of *B. cinerea* grow quickly, reaching 6.0 cm diameter and more in 10 days at 20 °C on oatmeal agar, at first hyaline but later becoming grey to greyish brown (Domsch et al. 1980). Conidiophores arise irregularly and often in patches, without a basal swelling, generally 2 mm or more long, mostly 16–30 µm thick, branched, often with a stipe and a rather open head of branches, smooth, clear, brown below, paler near the apex and with the ends of the branches often quite colourless. The conidia are ellipsoidal or obovoid, often with a slightly protuberant hilum, colourless to pale brown and smooth (Ellis and Waller 1974). Sclerotia are black and usually smaller and thinner than those of *Sclerotinia sclerotiorum*. *B. cinerea* is distributed worldwide, but occurs mainly in humid temperate and subtropical regions (Domsch et al. 1980). Ellis and Waller (1974) provided a description of *B. fabae*. Conidiophores are not normally found on leaves under field conditions but develop and produce conidia in a humid chamber. In culture on bean leaf agar conidium production is encouraged by the presence of relatively high concentrations of inorganic salts such as sodium nitrate. Sclerotia are formed abundantly in culture, discrete or sometimes confluent and mostly 1–1.7 mm in diameter, rarely up to 3 mm. When the weather turns dry and the infected plants are disturbed, clouds of spores are released into the air. Flowers can show symptoms of infection with typically grey mouldy growth present on petals, causing flower death (Bayaa and Erskine 1998). The infected pods are covered with the grey mouldy growth, rot and turn brown when dried out. Seeds in these pods fail to fill properly (Davidson et al. 2004). Infected seeds are discoloured and shrivelled (Bayaa and Erskine 1998). When infected seeds are sown, seedling blight can occur. Seedling blight is characterized by the prolific grey mycelial growth of the pathogen on the hypocotyl at the soil line (Morrall 1997). This stage of the disease also has the potential to spread along seedling rows as the pathogen spreads from plant to plant (Morrall 1997), reducing seedling populations. There are several main sources of inoculum of *Botrytis* grey mould; these include seed-borne inoculum, sclerotia and mycelium in old infected trash and alternate host plants. Under Canadian conditions, the disease has been found to be highly seed-borne and can affect seed viability, seedling emergence and crop establishment (Morrall 1997).

Botrytis ciñerea has also been isolated from lentil seed in India (Rajendra et al. 1987), Spain (Diaz and Tello 1994) and the United States (Kaiser 1992). Sclerotia are considered the main survival structure for both *B. cinerea* and *B. fabae*. Sclerotia have the ability to produce conidia over an extended period of time. Resting mycelium in old host plant debris may survive and produce conidia under humid conditions for extended periods (Bayaa and Erskine 1998). The development of *Botrytis* grey mould epidemics is largely determined by the prevailing environmental conditions during periods of inoculum production and dispersal in the presence of the host. The coinciding of all these events can result in the development of an epidemic very quickly when compared to most other diseases (Jarvis 1980a). It is generally assumed that for *B. cinerea*, inoculum is always present in the field and that production, liberation and dispersal of inoculum are an ongoing process (Jarvis 1980b); for *B. fabae* this principle will not always apply given its restricted host range. Environmental conditions and canopy density have also been shown to be primary factors that influence the development of *Botrytis* grey mould epidemics in lentil crops (Kaiser 1992; Morrall 1997; Bailey et al. 2000). A dense crop canopy, especially following canopy closure, and humid conditions following rain favour the sporulation and dispersal of *B. cinerea* on decaying lentil tissue (Kaiser 1992), and its appearance is often characteristic of a lentil crop with rank growth (Morrall 1997). *B. cinerea* is known to have very broad host range covering more than 200 host plant species including many ornamental, horticultural, field crop and weed species; and host crops among legumes include faba bean, chickpea, field pea, lupin, lucerne, clover etc. (Jarvis 1980a). This broad host range provides the pathogens with a wide geographic distribution and alternate host mechanism, which plays an important role in its survival from one season to the next (Davidson et al. 2004).

18.7.3.4 Management

Practices that have been effective in crop canopy management can be used to avoid the creation of a microclimate which encourages disease epidemics (Bretag and Mateme 1998). Practices that delay or avoid the formation of a dense canopy include the adjustment of seed rates, use of wider row spacing to increase air flow, weed control and optimum fertilizer use, particularly avoiding high nitrogen levels (Bayaa and Erskine 1998; Lindbeck et al. 2002). A program of stubble reduction may also be undertaken by grazing, burning or burying, to reduce the carryover of infected stubble into the following season. In addition, potential alternate host plants can be controlled to reduce the early build up of disease inoculum (Lindbeck et al. 2002). Also lentils should not be grown adjacent or into a lentil, faba bean, chickpea, vetch or lathyrus stubble (Lindbeck et al. 2002). Farmers can reduce the risk of seedling blight and disease carryover by retaining seed only from disease free crops for sowing the crop in next season. Seed treatments with fungicides such as benomyl, carboxin, chlorothalonil or thiabendazole can reduce seed-borne inoculum levels (Morrall 1997; Bayaa and Erskine 1998; Lindbeck et al. 2002). Iqbal et al. (1992) evaluated 14 fungicides and found that benomyl, thiabendazole and tridemorph were most effective against *B. cinerea;* however, Bayaa and Erskine (1998) stated that fungicide control for grey mould in lentil was uneconomic. Foliar

fungicides viz., Carbendazim, Chlorothalonil, Mancozeb and Procymidone are recommended and are widely used products in Australia for control of *Botrytis* grey mould in lentil crops (Lindbeck et al. 2002, 2003). Resistance to *Botrytis* grey mould is poorly understood, but requires a better understanding to enable different sources of resistance to be utilized and subsequent pyramiding of resistance genes (Tivoli et al. 2006). Resistant lentil germplasm has been identified in Australia (Bretag and Materne 1999; Lindbeck et al. 2003), Canada (Kuchuran et al. 2003), Nepal (Karki 1993) and Pakistan (Tufail et al. 1993; Erskine et al. 1994).

18.7.4 Lentil Rust

18.7.4.1 Distribution and Economic Importance

It is regarded as the most important foliar disease of lentil (Erskine et al. 1994). Complete crop failures can occur due to this disease (Beniwal et al. 1993). Rust of lentil is widespread globally; but is considered to be a production problem in Algeria, Bangladesh, Canada, Ethiopia, India, Italy, Morocco, Pakistan, Nepal, Syria and Turkey (Erskine et al. 1994). The disease also occurs widely in South America including Argentina, Brazil, Chile, Colombia, Ecuador and Peru (Bascur 1993).

18.7.4.2 Symptomatology

Rust starts with the formation of yellowish-white pycnidia and aecial cups on the lower surface of leaflets and on pods, singly or in small groups in a circular form (Agarwal et al. 1993). Later, brown uredial pustules emerge on either surface of leaflets, stem and pods. Pustules are oval to circular and up to 1 mm in diameter. They may coalesce to form larger pustules (Bayaa and Erskine 1998). The telia, which are formed late in the season, are dark brown to black, elongated and present mainly on branches and stems. In severe infections, leaves shed and plants dry prematurely (Bakr 1993), without forming any seeds in pods or with small shrivelled seeds. The plant has a dark brown to blackish appearance, visible in affected patches of the paddock or in the whole paddock if totally infected (Beniwal et al. 1993). The disease generally starts from low-lying patches in the paddock and radiates towards the border (Bayaa and Erskine 1998). Lentil seed may be contaminated with pieces of rust-infected leaf, stem and pericarps, which can act as primary inoculum for the recurrence of the disease in most years (Khare 1981; Agarwal et al. 1993).

18.7.4.3 Aetiology

This disease is caused by *Uromyces viciae-fabae* (Pers.) Schroet. Rust is an autoecious fungus, completing its life cycle on lentil. The aecia of *U. viciae-fabae* are amphigenous or hyphyllous, usually in groups surrounding the pycnia or sometimes scattered, cupulate. The aeciospores are spheroidal, wall hyaline and verrucose. Uredia are amphigenous and on the petioles and stems, scattered, cinnamon in colour. Uredospores are ellipsoidal or obovoidal with wall very finely echinulate; pores are 3–4, equatorial or occasionally scattered on *Lathyrus*. Telia are like the uredia but black and larger. Teliospores are ellipsoidal, obovoidal or cylindrical,

rounded or subacute above; wall chestnut, smooth, thick at the sides; and pedicels sienna to luteous (Laundon and Waterson 1965). Rust may also perpetuate on weed hosts from where it may infect lentil crops by wind-borne teliospores. High humidity and cloudy or drizzly weather with temperatures 20–22 °C favour disease development (Agarwal et al. 1993). The disease generally occurs during the flowering/early podding stage. Aeciospores germinate at 17–22 °C and infect other plants forming either secondary aecia at temperatures of 17–22 °C or uredia at 25 °C. Uredosori develop later in the season and are rapidly followed by telia (Beniwal et al. 1993). After harvest, aecia and uredia present on lentil trash die out, but teliospores tolerate high temperatures and allow the fungus to survive the summer. At lower temperatures, uredospores could be an important means of survival (Bayaa and Erskine 1998). Uredomycelium is highly resistant to heat and sunlight and is probably important for continued development and survival of rust in hot, dry conditions. The predominant form of survival will vary with the environment and location (Bayaa and Erskine 1998). Teliospores germinate at 17–22 °C without a resting period and cause new outbreaks of the disease each season. There are 70 recorded hosts of *U. viciae-fabae* including lentil, chickpea, field pea, *Lathyrus* spp. and *Vicia* spp. (Parry and Freeman 2001). Degrees of host specialization and pathogenic variability do exist within populations of *U. viciae-fabae* worldwide.

18.7.4.4 Management

Cultural control methods currently recommended for control of *U. viciae-fabae* include control of volunteer plants over summer, isolation of new season crops from old host crop stubbles (MacLeod 1999) and destruction of old lentil stubbles (Prasad and Verma 1948). Early studies on the control of lentil rust in India found seed treated with Agrosan (phenylmercury acetate) to control seed-borne inoculum (Prasad and Verma 1948). Singh (1985) found Vigil (diclobutrazole) applied as a seed dressing prevented the appearance of *U. viciae-fabae* up to 70 days following inoculation with uredospores; bayleton (triadimefon) prevented disease appearance up to 40 days postinoculation and the untreated control was severely infected with rust 35 days after inoculation. Agarwal et al. (1976) found foliar applications of Hexaferb (Ferric dimethyldithiocarbamate) and Dithane M-45 to give the best control of *U. viciae-fabae* in experimental plots at Jabalpur, India. In addition, Dithane M-45 also increased plot yield by 82% and grain weight by 24% when compared to the untreated control. The use of host plant resistance is the best means of rust control (Bayaa and Erskine 1998). Genetic differences among genotypes and sources of resistance have been reported worldwide, with several rust-resistant lines available. Resistance to rust is reported to be controlled by a single dominant gene (Sinha and Yadav 1989). During their studies on factors influencing the mechanism of resistance to rust in lentil, Reddy and Khare (1984) reported that resistant cultivars contained more leaf surface wax, F, K, S, Zn, Fe, Cu and levels of phenols than susceptible cultivars which had higher levels of amino acids, N, Mn and sugars. Structurally there were no significant differences found between resistant and susceptible cultivars.

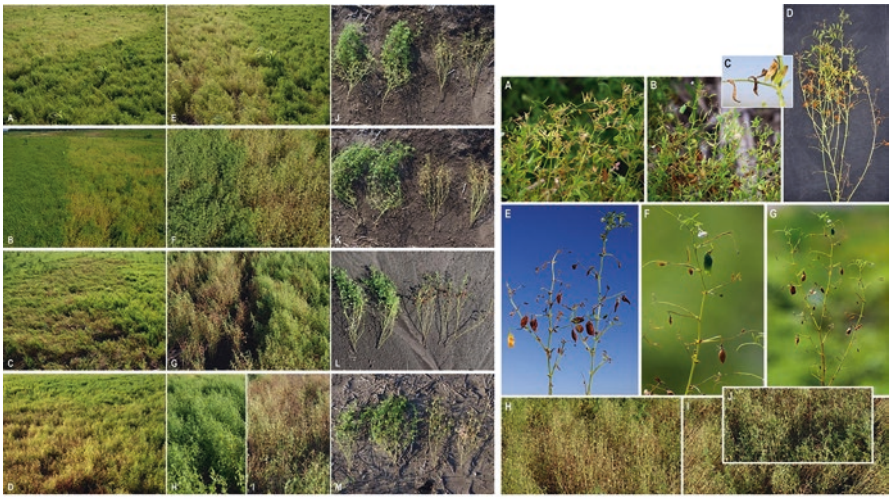

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Fig. 18.15 *Stemphylium* blight of lentil

18.7.5 *Stemphylium* Blight

18.7.5.1 Distribution and Economic Importance

The disease has been reported on lentil from Bangladesh (Bakr 1993), Canada (Morrall 2003), Egypt, Syria (Bayaa and Erskine 1998) and the United States (Wilson and Brandsberg 1965). The disease has the potential to cause yield losses of up to 62% under conducive conditions (Bakr 1993).

18.7.5.2 Symptomatology

Stemphylium blight start with the appearance of small pin-headed light brown to tan coloured spots on leaflets. These small spots enlarge rapidly under favourable weather conditions, covering the entire leaflet surface within a period of 2–3 days (Fig. 18.15). The infected tissue appears light cream in colour, often with angular patterns of lighter and darker areas that spread across, or long, the entire leaflet (Morrall 2003) (Fig. 18.15). The affected foliage and stems gradually turn dull yellow, giving a blighted appearance to the crop (Bakr 1993). The infected leaves can be abscised rapidly, leaving only the terminal leaflets on the stems. The stems bend down, dry and gradually turn ashy white, but pods remain green (Fig. 18.15). White mycelial growth can sometimes be seen on the infected stems (Bakr 1993). Important sources of *S. botryosum* inoculum include infected crop debris and infected seed. Infected crop debris can be a source of primary inoculum in the form of air-borne ascospores or as resting mycelium, based on the studies of the pathogen on other host crops such as alfalfa (Gilchrist 1990) known to be carried on seed (Booth and Pirozynski 1967),

and *Stemphylium* spp. has been isolated from lentil seed in Australia (Nasir and Bretag 1997a, b), but the significance of seed-borne *S. botryosum* inoculum on disease initiation in lentil is not clearly understood (Mwakutuya 2006).

18.7.5.3 Aetiology

The disease is caused by *Stemphylium botryosum* Wallr (Pleosporales, Pleosporaceae) (teleomorph: *Pleospora herbarum* (Fr) Rab:). Conidiophores of *S. botryosum* have 1–7 septata, are pale brown to brown, have a swollen apical sporogenous cell and are slightly roughened toward the apex. They possess a single apical pore. Conidia are oblong, olive to brown, ovoid to subdoliiform, occasionally constricted at 1–3 transverse septa and at the 1–3 longitudinal septa if complete, with a single basal pore and a roughened outer wall. Ascostromata are scattered, immersed to erumpent in the tissue of the host. Asci contain eight ascospores, cylindrical to slightly club shaped. Ascospores are light to yellow brown, ellipsoid to club shaped with seven septa, slightly constricted at the three primary transverse septa and muriform (Booth and Pirozynski 1967). Bakr (1993) has reported from Bangladesh that the pathogen commences infection when the ambient night temperature remains above 8 °C and the mean day temperature exceeds 22°C. The RH inside the crop canopy must also reach 94%. In India, Sinha and Singh (1993) found that an average mean temperature of 18 °C ± 2 °C and RH of 85–90% in the morning was favourable for disease development and spread. Most recently, in Canada Mwakutuya (2006) found that symptom development of *S. botryosum* was optimized after 48 h of leaf wetness at temperatures above 25 °C. The host range of *S. botryosum* is wide and includes a large number of ornamental, horticultural and field crop species. These include lentil (Bakr 1993), lupin (Tate 1970), tomato (Bashi and Rotem 1975) spinach (Koike et al. 2001), alfalfa, clover (Smith 1940); lettuce (Tate 1970), apple, onion and gladiolus (Booth and Pirozynski 1967).

18.7.5.4 Management

There is little published information available regarding cultural control methods for *S. botryosum* in lentil. Being stubble-borne, strategies such as destruction of old crop residues, and crop rotation would assist in decreasing potential inoculum sources. In Bangladesh, delayed sowing was found significant in reducing the incidence of *Stemphylium* blight in lentil, but it decreases the crop yields (Bakr 1993). Foliar fungicides have been found to be effective in the management of *Stemphylium* blight. Application of Royal 50 WP was found to effectively control the disease when applied three times at weekly intervals starting from the initiation of the disease (Bakr 1993). In other horticultural crops, such as asparagus and garlic, the disease has been successfully controlled using chiorothalonil (Meyer et al. 2000), tebuconazole and procymidone (Basallote-Ureba et al. 1998). Sources of host plant resistance have been identified in screening nurseries in Bangladesh. The resistant varieties ‘Barimasur 3’ and Barimasur 4′ were released with resistance to *S. botryosum* (Sarker et al. 1999a, b). Studies by Chowdhury et al. (1997) found lentil cultivars

Fig. 18.16 Anthracnose of lentil

with resistance to *S. botryosum* had a higher number of epidermal hairs, thicker cuticle, thicker epidermal cell layer and thicker cortical layers. In addition, *S. Botryosum*-resistant lines were also found to have fewer stomata than susceptible cultivars.

18.7.6 Anthracnose

18.7.6.1 Distribution and Economic Importance

It is reported from Bangladesh, Canada, Ethiopia, Morocco, Syria and Canada (Kaiser et al. 1998; Anderson et al. 2000). The Australian lentil industry remains under threat from this exotic disease (Ford et al. 2004).

18.7.6.2 Symptomatology

Lesions on leaves are circular with few acervuli in the middle of each lesion and premature leaf drop begins at early flowering (Fig. 18.16). Conidia form in acervuli on infected plants, and secondary spread of conidia to neighbouring plants occurs by rain splash. The fungus penetrates the vascular tissue, which results in plant wilting, and large brown patches of dying plants become evident in the field after flowering (Fig. 18.16). The disease is favoured by high humidity and temperatures of 25–30 °C and is seed-borne. The pathogen is capable of surviving for up to 4 years as microsclerotia in crop residue and may become active again when in contact with fresh host tissue, spreading as conidia with rain splash and on plant debris through wind dispersal between crops (Buchwaldt et al. 1996).

18.7.6.3 Aetiology

This disease is caused by *Colletotrichum truncatum* (Schwein.) Andrus and Moore. In culture, the mycelium of *C. truncatum*, growing on PDA at 25°C, is dark brown to black in colour with setae being rare. The conidia are ellipsoidal, hyaline and aseptate with one rounded and one pointed end. Setae are generally acicular, swollen at the base, tapered to the apex and comprised of one or two septa. Mycelial appressoria and appressoria produced directly from the germtube are generally brown, clavate and occasionally irregular. No teleomorph has been found in the nature (Kaiser et al. 1998); however, the teleomorph was recently induced under laboratory conditions and named *Glomerella truncata* (Armstrong-Cho and Banniza 2006) and had a blackish brown appearance. A study on the infection process of leaves inoculated with a spore suspension by Chongo et al. (2002) found that in the initial infection phase, conidia germinated within 3–6 hours after infection (HAI) and formed appressoria at 6–12 HAI. By 24 hours after infection, hyphae infected epidermal cells inter- and intracellularly. Differential host cell reaction was observed by the resistant cultivar 2–3 days after infection. Hyphal spread was slower and phenolic compounds accumulated more quickly in the resistant line, resulting in fewer, smaller lesions than in the susceptible cultivar.

18.7.6.4 Management

The current disease management practices are based primarily on application of foliar fungicides such as chlorothalonil or benomyl (Chongo et al. 2002). However, seed treatment with fungicides such as benomyl or thiabendazole provides complete control of the seed-borne fungus. Breeding for resistance has suffered from a lack of highly resistant germplasm to include in breeding programs. In Canada, Buchwaldt et al. (2004) found only 16 out of 1771 accessions of lentil resistant to anthracnose after field and glasshouse screening. As well two pathotypes of *C. truncatum* were identified with the Ct0 pathotype isolated more frequently from commercial seed samples than the Ct1 pathotype, although both pathotypes were isolated with similar frequency from plants in commercial fields planted with susceptible cultivars. Pathotype Ct0, to which no resistance has yet been identified, presents a high risk to lentil production potentially worldwide. PCR-based diagnostics tests have been developed to detect the pathogen in plant tissues (Ford et al. 2004) and are a valuable and reliable alternative to conventional seed health testing methods. These tests can be applied directly to suspected infected tissues taken from the field, to identify the pathogen much faster and potentially more accurately than traditional culturing techniques.

18.8 Field Pea (*Pisum sativum* L.)

Field pea or ‘dry pea’ is a cool-season legume crop, which is commonly used throughout the world in human diet. Field pea has high levels of amino acids, lysine and tryptophan, which are relatively low in cereal grains. Field pea contains approximately 21–25% protein. Peas contain high levels of carbohydrates, are low in fibre

and contain 86–87% total digestible nutrients, which makes them an excellent component of human diet or livestock feed. Pea is highly susceptible to pre-emergence damping off and to post-emergence root and foot rots caused by soil-borne and seed-borne fungal infection.

18.8.1 Ascochytosis

18.8.1.1 Distribution and Economic Importance

Ascochytosis in peas is a problem in many parts of the world including some parts of India (Agarwal and Nene 1987). There are three species of *Ascochyta* associated with the ascochytosis of peas. The losses caused by *Mycospherella pinodes* and *A. pinodella* are more severe than *A. pisi* (Wallen 1965).

18.8.1.2 Symptomatology

The symptoms induced by these pathogens are almost similar. All parts of the plant are affected. On leaves, circular spots of tan to brown colour with a dark-brown margin appear. Similar spots also appear on pods but they are shrunken. On stem, lesions are elongated, sunken and purplish-black in colour. These lesions coalesce and weaken the stem by girdling the entire stem. Sometimes the stem may also break of (Walker 1957).

18.8.1.3 Aetiology

There are three fungal species viz., *Ascochyta pisi* Lib.; *A. pinodella* Jones; *Mycosphaerella pinodes* (Berk. & Blox.) Stone] associated with the ascochytosis of peas. All the pathogens are internally seed-borne, the infection being deepseated in the form of mycelium (Babak and Balashova 1962; Wallen 1965; Maude 1966). Infected plant debris left in the soil also serves as a source of inoculum.

18.8.1.4 Management

Seed treatment with Thiram or Captan at 0.25% or Benomyl at 0.4% against *A. pisi* and *M. pinodes* (Parfilov 1958; Maude and Kyle 1970; Yoshii 1975). Soaking seed for 24 h in 0.2% suspension of Thiram has also been found effective (Maude 1966). A crop rotation of 3 years reduces chances of soil infection (Agarwal and Nene 1987).

18.8.2 Bacterial Blight

18.8.2.1 Distribution and Economic Importance

Bacterial blight of pea was first described from Colorado, U.S.A. in 1916 (Walker 1952). This disease occurs worldwide. It was formerly common, but now appears only occasionally (Hagedorn 1991).

Fig. 18.17 Bacterial blight of field pea

18.8.2.2 Symptomatology

Lesions appear on all above-ground plant parts. They begin as small, water-soaked, oval spots. Individual leaf and stipule lesions enlarge to approximately 3 mm in diameter with an angular shape (Fig. 18.17). Pod lesions are 6 mm or more in width, and stem lesions are long and resemble stem streaks, which turn brown with age. Lesions on foliage generally turn brown, shiny and translucent. Pod lesions, which may expand along either suture, slowly change from a water-soaked to greasy appearance with purplish-brown margins (Fig. 18.17). Seeds inside infected pods may develop brown discolouration (Fig. 18.17). Multiple lesions often coalesce to cover large portions of infected plants. This may cause plants to look 'blighted' and dry prematurely. There are four known races of the causal bacterium *Pseudomonas syringae* pv. *pisi* of this pathogen. The bacterium overwinters in infected plant debris and in the seed coat. Splashing water spreads the disease locally; infected seed spreads the disease long distance.

18.8.2.3 Aetiology

This disease is caused by bacterium *Pseudomonas syringae* pv. *pisi* (Sackett) Young, Dye & Wilkie [Syn. *Pseudomonas pisi* (Sackett)]. The bacterium is reported to be seed-borne. In addition, it may also survive in infected crop debris in soil. Bacterial blight has been found in peas grown after beans infected with bacterial brown spot. It occurs first in localized areas in a field if introduced via the seed. If the primary inoculum is from overwintered plant debris, disease may be widespread throughout the field. The bean bacterial brown spot pathogen, *Pseudomonas syringae* pv. *phaseoli*, can also attack peas causing bacterial blight symptoms (Walker 1952). Environmental factors such as cool, overcast weather with high humidity promotes disease development. Warm, dry conditions slow the disease (Sackett 1916; Ludwig 1926; Skoric 1927; Hagedorn and Wade 1964; Taylor 1972).

18.8.2.4 Management

Use of certified seed. Seed treatment with Streptomycin sulphate at the rate of 2.5 g a.i. per kg of seed reduces seed-borne inoculum. Destruction of infected crop debris (Agarwal and Nene 1987).

18.8.3 *Ascochyta* Diseases

Mycosphaerella Blight, *Ascochyta* Foot Rot, *Ascochyta* Leaf and Pod Spot. *Mycosphaerella pinodes* (Berk. & Blox).- the perfect stage of *Ascochyta pinodes*, which causes blight; *Ascochyta pisi* Lib., which causes leaf and pod spot and *Phoma medicaginis* var. *pinodella* (Jones), which causes foot rot.

18.8.3.1 Distribution and Economic Importance

Ascochyta diseases occur worldwide and are occasionally economically significant when cropping procedures and environmental conditions favour disease development. *Mycosphaerella* blight is the most important of the three *Ascochyta* diseases of pea (Hagedorn 1991).

18.8.3.2 Symptomatology

Seed infestation can result in high levels of disease in subsequent crop. Seed-borne infestation by *M. pinodes* and *P. pinodella* usually causes more widespread and severe seedling diseases than *A. pisi* infestation (Bowen 1992). Infected seeds show varying degrees of shrivelling and discolouration, while some infected seeds remain symptomless. Planting of *Ascochyta*-infected seeds reduced number or vigour of emerging plants and similarly had deleterious effects on yield (Hwang et al. 1991; Tivoli et al. 1996; Marcinkowska et al. 2009; Setti et al. 2009; Boros and Marcinkowska 2010). *P. pinodella* and *M. pinodes* resulted in black coloured epicotyls and slight plant weight reduction (Persson et al. 1997). These pathogens were prevalent in most pea crops in South Australia and were identified as probable major contributors to yield decline (Davidson and Ramsey 2000). Pre-emergence damping off and post-emergence foot rot are characteristic symptoms of seed-borne *M. pinodes* (Moussart et al. 1998). Infection caused by *M. pinodes* and *P. pinodella* produces indistinguishable symptoms that include stem base rot as well as necrotic spots on leaves, stems and pods. Leaf and pod spots are usually caused by *A. pisi* (Wallen 1965, 1974).

18.8.3.3 Aetiology

Three fungal species *Ascochyta pisi* Lib., *Mycosphaerella pinodes* (Berk. and A. Bloxam) Verstergr (anamorph *Ascochyta pinodes* L.K. Jones) and *Phoma pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch (syn. *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema) are responsible for *Ascochyta* blight disease of pea. All species can be seed-borne and survive on plant residues in soil. They occur singly or in combination and are referred to as the *Ascochyta* complex (Wallen 1965; Onfroy et al. 1999). Identification by cultural and morphological characteristics (size of pycnidia, shape and size of conidia, and presence or absence of chlamydospores, may be uncertain because it is based on small differences in morphological criteria that are often dependent on cultural conditions, leading to misidentification (Faris-Mokaiesh et al. 1996). *M. pinodes* was distinguished from *P. pinodella* on the basis of presence of pseudothecia, a higher number of larger, bicellular conidia, compared with the smaller, predominantly unicellular conidia of *P. pinodella* (Onfroy et al. 1999). The lesions caused by *A. pisi* are different from those caused

by *M. pinodes* and *P. pinodella*, both of which cause substantial losses in yield and seed quality (Allard et al. 1993). Apart from *Ascochyta* complex, seed-borne fungi are of *Fusarium* genus, *Alternaria tenuissima*, *A. alternata*, *Penicillium* spp. and other fungi that can also cause root rots and deteriorate pea seedlings' germinating power, growth and health (Prokinova and Markova 1997; Marcinkowska 2008).

18.8.3.4 Management

Development of pea cultivars resistant to main causal agents of root rots would assist in controlling this disease and minimizing its damage. Partial resistance to *M. pinodes* in pea is quantitatively inherited (Prioul-Gervais et al. 2007). However, partial resistance was not expressed when pathogen inoculum concentration was high (Onfroy et al. 2007). Whereas high levels of resistance to Ascochyta blight have not been found, the most effective practices could be an integrated disease management (Davidson and Kimber 2007). Fungicidal seed treatment prevents transmission of seed-borne fungal pathogens and also controls soil-borne fungi which induce seed rots and pre- and postemergence root and stem disease of young seedlings, causing poor seedling establishment. Evidence is available that crop rotation, destruction of infected pea trash and chemical seed treatments can significantly reduce the amount of primary inoculums (Bretag et al. 2006). Effective seed treatment managed to reduce seed-borne inoculum and lessened introduction of the pathogens into the new areas (Hwang et al. 1991).

18.8.4 *Mycosphaerella* Blight

Small, purple spots develop on the surface of the foliage, stems and pods (Fig. 18.18). On leaves, the purple spots may enlarge to 5–6 mm in diameter and turn dark brown to black. Small lesions usually have no definite shape or margin, but larger lesions are circular with distinct margins and opaque centres. Leaves and stipules with many lesions turn strawcoloured, dry out and die. Dead leaves remain attached to the plant. Pinpoint-sized pod lesions are similar to those on foliage, and they may

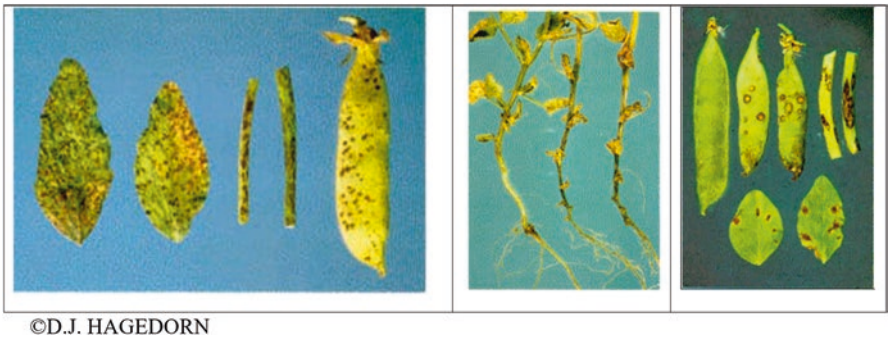

Fig. 18.18 *Ascochyta* diseases of field pea

coalesce and enlarge into sunken, oval lesions with dark margins. Severely infected young pods wither, thereby lowering the processing quality of the peas.

18.8.5 *Ascochyta* Foot Rot

This disease is similar in appearance to *Mycosphaerella* blight on leaves and foliage, although it is often most apparent on the lowest leaves and stipules and on the lower stem and upper root. Purple to blue-back lesions up to 1 cm in length extend along the stem (Fig. 18.18). These lesions often coalesce and girdle the stem, giving it a blue-black look. Severe stem infections may cause the entire plant to mature rapidly and prematurely.

18.8.6 Leaf and Pod Spot

This disease has lesions distinct from *Mycosphaerella* blight and *Ascochyta* foot rot. Typical lesions on foliage and pods are larger 5–8 mm in diameter and are sunken and tan in colour, with a dark and distinct border (Fig. 18.18). Lesions are oval on foliage and pods, elongated on stems. The centres of lesions caused by all three pathogens show dark pimple-like structures containing the easily dispersed spores which spread the disease (Fig. 18.18).

Spread Three fungi, *Mycosphaerella pinodes*, *Phoma medicaginis* var. *pinodella* and *Ascochyta pisi*, incite *Ascochyta* diseases of peas. The pathogens are carried in infected seeds and overwinter in infected plant debris. Spores originating from overwintered plant debris spread in two ways. The first, in early spring, is by asexual conidia which depend upon water for discharge and dissemination. Wind-blown rain is an important carrier. The second, in early June in northern states, is by sexually formed ascospores which are forcibly discharged into the air and then carried by air currents for 0.4 km or more. Ascospores disseminate the disease more widely than do conidia.

Environmental Factors Disease development is favoured by high moisture conditions (repeated rain and high humidity) and can take place over a range of temperatures (Jones 1927; Hare and Walker 1944; Wallen 1974).

18.8.7 Anthracnose

18.8.7.1 Distribution and Economic Importance

Pea anthracnose has been reported from several pea-producing areas around the world, but is only seen rarely and is generally of minor importance (Hagedorn 1991).

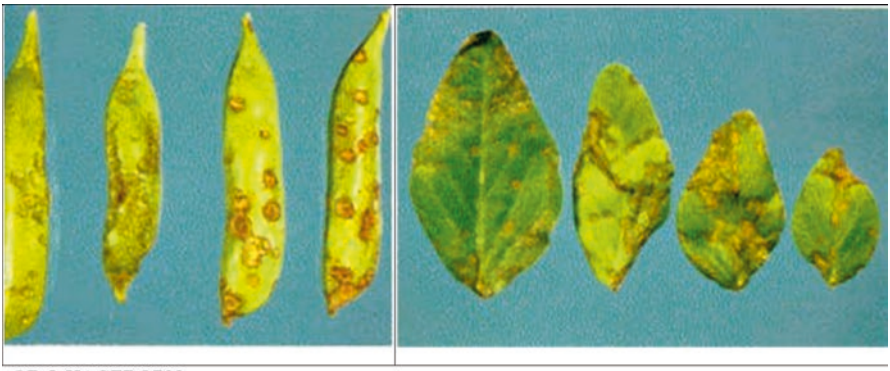

Fig. 18.19 Anthracnose of field pea

18.8.7.2 Symptomatology and Aetiology

Leaf and stipule lesions are oval, 2–8 mm in diameter and have brown margins with grey-tan centres (Fig. 18.19). Lesions on the stem are long and similar in colour to those on the leaves. Grey-tan pod lesions are round and sunken, with reddish-brown borders (Fig. 18.19). These are most dramatic when they form on immature pods, causing them to develop abnormally and show brown discolouration. The fungus is not an aggressive pathogen. Severe disease symptoms, especially on stems, are often associated with wounds or *Ascochyta* infections. The fungus survives from one growing season to the next on infected plant debris and on seed, although seed transmission is extremely slight. Spattering and wind-blown rain are the primary means of local dissemination of the infectious spores.

18.8.7.3 Aetiology

This disease is caused by a fungus *Collectotrichum pisi* (Pat). Frequent wind-blown rain, high humidity and warm temperatures are most conducive to disease development (Jones and Vaughan 1921; Ou and Walker 1945; Hagedorn 1974).

18.8.8 *Septoria* Blotch

18.8.8.1 Distribution and Economic Importance

Septoria blotch, though a common disease in the Midwest, is rarely significant because it occurs primarily on old foliage, pods and stems (Hagedorn 1991).

18.8.8.2 Symptomatology

Disease lesions are yellow areas of varying size and shape with no definite margins (Fig. 18.20). They are often so numerous that they cover whole leaves or stipules. In these cases, the affected foliage may dry out, giving the plant a prematurely aged look. As the blotches dry out, many pinpoint-sized black pycnidia (fungus fruiting bodies) may be seen scattered widely on the infected plant parts, including pods.

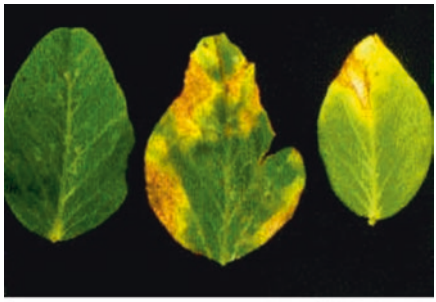

Fig. 18.20 *Septoria* blotch of field pea

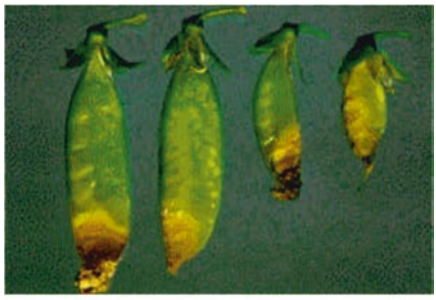

Fig. 18.21 *Botrytis* (grey) mould of field pea

The organism overwinters on infected plant debris. Spattering water promotes field spread. Seed transmission may occur, but little is known about this phenomenon, and it is not considered important. Environmental factors favouring diseases development are prolonged high humidity (at least 24 h) and moderate temperatures of 21–27 °C (Melhus 1913; Cruickshank 1949).

18.8.8.3 Aetiology

The disease is caused by *Septoria pisi* and *Septoria flagellitera* (West)

18.8.9 *Botrytis* (Grey) Mould (*Botrytis cinerea* Pers.ex Fr)

18.8.9.1 Distribution and Economic Importance

Botrytis (grey mould) is a common and troublesome disease of peas in northern Europe and occurs occasionally in the more humid pea-growing areas of the United States (Hagedorn 1991).

18.8.9.2 Symptomatology and Aetiology

The most striking and economically important symptoms are on the pod, although foliage and stem infections may also occur (Fig. 18.21). Pod lesions usually occur at the terminal end, where adhering blossom parts provide an excellent medium for

rapid saprophytic growth of the fungus (Fig. 18.21). The fungus then spreads upward into the young pod and forms an oval or semi-circular lesion up to 2 cm in diameter. Initially these lesions are water-soaked, but soon turn grey from many fruiting structures which develop on the lesion surface. Occasionally kernel-like, black, hard sclerotia of the fungus are formed in the older decayed tissue. The pathogen is common and widespread because it can live and grow on dead or decaying organic materials. Spores are formed prolifically on the surface of infected organic matter and are readily airborne. *Botrytis cinerea* can infect pea plants by spores that have been blown or splashed onto blossoms or by mycelium growing onto leaves touching the debris. Any activity which spreads infected plant debris also spreads the fungus. Moderate temperatures (16–21 °C) and moist (nearly 100% relative humidity) conditions are favourable for disease development. Potassium deficiency before and during flowering may make the plant more susceptible (Ford and Haglund 1963; Wijngaarden and Ellen 1968).

18.8.10 Near-Wilt and Other Pea Wilts

18.8.10.1 Distribution and Economic Importance

Near-wilt was a problem primarily in the Midwest between 1930 and 1960, but is now of lesser importance due to the availability of resistant cultivars. Wilt is present throughout the United States, but is rarely a problem because almost all cultivars are resistant. Race 5 and race 6 wilts are known to be troublesome only in western Washington state. Near-wilt diseased plants are scattered throughout the field, rather than occurring in localized patches. In contrast, plants infected with pea wilt appear in localized spots in the field (Hagedorn 1991).

18.8.10.2 Symptomatology

Initial symptoms of near-wilt begin about late blossom/first pod stage. Foliage loses its normal green colour and the paler leaves and stipules curl downward (Fig. 18.22). These symptoms often appear on one side only and wilting may not extend to the other side of the plant until the wilting process is well-advanced. As the plant wilts, it gradually turns a tannish-straw colour (Fig. 18.22). Diseased plants are stunted. Internally they show a brick-red discolouration of the vascular system which may extend to the top of the plant (Fig. 18.22). Generally, there are no external symptoms on the lower stem and upper taproot, but occasionally the cortical tissue may be slightly decayed. Infected plants eventually die. Near-wilt is most common on mid- or late-season cultivars. Pea wilt attacks young plants. Early maturing cultivars may be affected and show yellow-orange internal discolouration in the lower internodes.

18.8.10.3 Aetiology

Fusarium oxysporum Schl., f. sp. *Pisi* (Van Hall Snyd. & Hans). These fungi can live indefinitely in the soil, with or without the presence of peas. Local spread of the fungus may be by water or farm implements moving infested soil or infected pea vines. Infected seed can transmit the fungus over long distances. Near-wilt occurs

Fig. 18.22 Near-wilt and other pea wilts of field pea

later in the growing season than the other wilts because the optimum temperature for disease development is higher: 26 °C for near-wilt and 21 °C for the other wilts (Linford 1928; Walker 1931; Snyder and Walker 1935; Hagedorn 1953; Haglund and Kraft 1970, 1979).

18.8.11 *Fusarium* Root Rot (*Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. and Hans)

18.8.11.1 Distribution and Economic Importance

Fusarium root rot is a troublesome and sometimes important pea disease in almost all of the pea-producing areas of the United States (Hagedorn 1991).

18.8.11.2 Symptomatology and Aetiology

Initial symptoms often appear near the seed area and consist of slender, light brown lesions along the taproot and on the side roots (Fig. 18.23). These lesions enlarge and coalesce until the main roots become completely covered, shrunken and dark brown or light red in colour (Fig. 18.23). This discolouration and shrinking continues upward to 2.5–5 cm above the soil line. The shrinking is caused by the collapsing of dead cortical cells. The vascular tissue also shows red discolouration extending upward one to three nodes above the soil line. Diseased plants variously dwarfed depending upon the severity of infection, and may wilt and die.

18.8.11.3 Aetiology and Disease Spread

The disease is caused by the soil-borne fungus *Fusarium solani* f.sp. *pisi*. Any activity which spreads soil also spreads the fungus. Relatively high soil temperatures of 25 °C and above are optimum for disease development. Soil moisture has little

Fig. 18.23 *Fusarium* root rot of field pea

influence, needing only to be within ranges conducive to good plant growth (Jones 1923; Hagedorn 1960; Lockwood and Ballard 1960; Kraft and Roberts 1970).

18.8.12 Seed-Borne Mosaic Disease

18.8.12.1 Distribution and Economic Importance

This is potentially important viral disease and has been found to be common in pea germplasm collections in many countries, including the United States. However, because of vigorous research on identifying the presence of the virus in pea seed and destruction of seed fields in which the virus is found, this disease has rarely caused substantial economic loss in commercial pea fields (Hagedorn 1991).

18.8.12.2 Symptomatology

Symptoms on pea seedlings include stunting and downward leaf curling (Fig. 18.24). Vein clearing and mosaic may also be present. Diseased plants continue to be stunted and appear malformed because of foliage curling, apical malformation and failure of internodes to elongate. Pods are often very short and stubby and contain few, if any, seeds. Seed coats of infected seeds may be cracked or show a necrotic line pattern (Fig. 18.24). The tendrils often curl into tight balls. Some plants may carry the virus but exhibit no symptoms. Rates of seed transmission of up to 30% have been reported. Several strains of the virus occur. The virus can infect at least 30 plant species (mostly legumes) but has a very limited host range in nature. It is

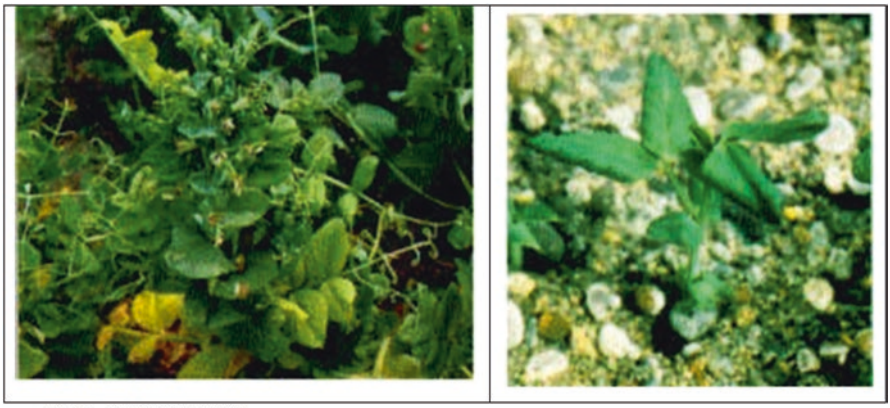

Fig. 18.24 Seed-borne mosaic disease of field pea

readily transmitted by several aphid species including the pea aphid. Normal temperatures and moisture are satisfactory for disease development.

18.8.12.3 Aetiology

The disease is caused by *Pea seed-borne mosaic virus.* Warm, dry weather, which encourages aphid population build-up, may aid virus spread in the field. Seed transmission may be suppressed by high temperatures (above 35 °C) (Stevenson and Hagedorn 1969, 1970; Stevenson et al. 1970; Hagedorn and Gritton 1973; Hampton and Mink 1975; Khetarpal and Maury 1987).

18.9 Rajmash/French Bean (*Phaseolus vulgaris*)

The seed and soil-borne pathogens *Phaeoisariopsis griseola* perpetuate through infected plant (*Phaseolus vulgaris*) debris from one season to the next, remaining viable under field conditions for 10 months. Viability of conidia on debris was 6 months in the lab and 8 months in the field. In seeds the fungus remained viable for 1 year as dormant mycelium, this being important in the initiation and spread of the disease in new localities (Sindhan and Bose 1979). Although *S.* (*Corticium*) *rolfsii* is an important pathogen of french beans, it has not previously been recorded on the *Phaseolus vulgaris* seed; it was isolated from two samples from Uganda and pathogenicity was established (Singh and Mathur 1974). French bean (*Phaseolus vulgaris*) seed samples were collected from different parts of Tamil Nadu, including both tropical and temperate regions, and cultivars Arkakomal, Premier, Rajmash DPU 88-4 and Watex. Watex had the highest percentage seed infection (70%) by *C. lindemuthianum* with more discolouration. Among the various techniques adopted for detection, the agar plate method was the best and yielded maximum percentage seed infection. Direct correlation was obtained between seed discolouration and

percent seed infection. The pathogen was present mostly in seed coats and cotyledons, and rarely in embryonic axes. Artificial inoculation of 45-day-old plants resulted in maximum disease incidence and seed infection, and minimum seed yield/plant and 100-grain weight (Ravi et al. 1999). The virus causing mosaic mottling and downward curling of leaf tips and margins of seedlings from tested seed was identified as cucumber mosaic cucumovirus on the basis of transmission, hosts and serological relationships. The extent of seed-borne infection varied with the cultivar. Seeds nearest to the pedicel were more frequently infected than others in the pod. Within the seed, the virus was concentrated in the embryo. The virus remained infective even in powder from crushed seed. This is the first report of CMV infecting *P. vulgaris* in India (Bhattiprolu 1991).

Pelleting of *Phaseolus vulgaris* seed with streptomycin sulphate for the control of seed-borne *Pseudomonas phaseolicola* was tested and compared with a streptomycin soak treatment. The failure of pelleting to control the infection is attributed to slow absorption of streptomycin from the pellet compared with its rapid uptake from solution (Ralph 1976). Khare et al. (1979) tested seven fungicide spray treatments; and Dithane M-45 (mancozeb) gave the best control of various leaf diseases of *Phaseolus vulgaris*, with maximum reduction in phylloplane fungi, and had the best effect on yield. Association of fungi with harvested seed was least with Dithane M-45 and Sulfex. Six species of *Trichoderma* (*T. hamatum, T. harzianum, T. koningii, T. pseudokoningii, T. longibrachiatum* and *T. viride*), *Gliocladium virens, Bacillus subtilis* and *Pseudomonas fluorescens* were tested for their antagonistic activity against seed-borne *Colletotrichum lindemuthianum* in *Phaseolus vulgaris. T. viride* recorded the maximum inhibition of mycelial growth followed by *P. fluorescens* and *T. harzianum* in a dual culture technique. The culture filtrate of *T. viride* exerted the maximum inhibition of pathogen spore germination and mycelial growth. Infected *P. vulgaris* seeds soaked in 10% culture filtrate, treated with 0.4% talc formulation of *T. viride* recorded minimum seed infection and maximum seed germination (Ravi et al. 1999).

18.10 Conclusion

The chapter includes major pulses and their seed-borne diseases. Damages caused by the seed-borne diseases are considerably high in some pulses; hence it is need of hour to care about the seed health. Seed is the richest source to be attracted by pests and diseases resulting into several seed-borne diseases. Use of clean seed is an important disease control measure because seed transmits plant pathogens and seed is exchanged worldwide. Each year, seeds are exchanged throughout the world by commercial seed trading activities, through germ plasm exchange activities of international organizations, and by public and private institutes concerned with crop improvement. Because many plant pathogens can survive in, on, or with seed, seed has been and continues to be an important vehicle for transmitting plant pathogens throughout the world. Thus, use and exchange of clean seed is an important method to manage plant disease.

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Major Seed-Borne Diseases in Important Vegetables: Symptomatology, Aetiology and Its Economic Importance

19

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Abstract

Diet without vegetables would lead to numerous nutritional malformations and physiological deformities in human health. However, despite its importance, uncountable seed-borne diseases pose a major threat causing economic yield losses and its reduction in production and productivity. The importance of the seed-borne diseases of vegetables has been ignored. Therefore, the prime need is to identify those seed-borne diseases caused by various biological agents like fungi, bacteria and viruses. These pathogens are inevitable guest menacing at different growth stages starting from emerging seedling stages to harvesting and continue till post-harvest interlude. They affect seed storability, physical appearance, viability and germinations. The proper identifications of these pathogens, their symptomatological appearance on the affected plants and their host pathogen relationship will definitely help us to formulate management strategies effectively that will ultimately increase the yield of the vegetables and improve the market quality. Therefore, our primary aim in this chapter is to discuss about the various causal agents for seed-borne diseases intruding in important vegetables and to give an idea about diverse competent control methods which includes cultural, physical, biological and chemical methods.

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19.1 Introduction

Vegetables are affluent and reasonably cheaper source of vitamins and minerals. Consumption of vegetables provides taste and palatability, increases appetite, provides fibre for digestion, prevents constipation and most important is providing plenty amount of protein. Some of the vegetables are good sources of carbohydrates (potato, onion and garlic), proteins (peas, beans, leafy vegetables and garlic), vitamin A (carrot, tomato, leafy vegetables), vitamin B (peas, garlic and tomato), vitamin C (green chillies, Cole crops, leafy vegetables) and minerals (leafy vegetables). As per dietician, daily requirement of vegetables is 75–125 g of green leafy vegetables, 85 g of other vegetables and 85 g of roots and tubers with other food. Many of the vegetable crops have high medicinal value for curing certain diseases. For example, onion and garlic are found to possess antibacterial property (Gebreyohannnes and Gebreyohannnes 2013). Many solanaceous and cucurbitaceous vegetables are found to possess vitamin D. India has been considered as self-sufficient in food production; however, to provide health security to our population, we need to have assurance for nutritious food through balanced diet. Vegetables form the most important component of a balanced diet, and vegetables can be grown round the year. India is the world's second largest producer of vegetables next only to China. India is a land of diverse agroclimatic conditions making it possible to grow a wide variety of vegetable crops round the year in one part of the country or another. As many as 61 annual and 4 perennial vegetable crops are commercially cultivated; some of them are very important vegetable crops (Table 19.1). Despite its importance, the vegetables have been subjected to many biotic and abiotic stresses. Among biotic stresses, seed-borne diseases caused by many fungal, bacterial and viral pathogens play a pivotal role in decreasing the production and productivity of the vegetables not only in India but also in other parts of the world. The diseases that have been discussed in this chapter are given in Table 19.2.

Table 19.1 Some important vegetables grown in different parts of India

Crop group	Important vegetable crop's name
Solanaceous crops	Brinjal, tomato, potato, chillies, potato
Cole crops	Cabbage, cauliflower
Okra	Okra
Cucurbits	Longmelon, muskmelon, snapmelon, watermelon, cucumber, pumpkin, summer squash, bitter gourd, bottle gourd, pointed gourd (parwal), ridge gourd, round gourd, snake gourd, sponge gourd, wax gourd (ash gourd)
Root vegetables	Carrot, radish, turnip
Leguminous vegetables	Broad bean, cluster bean, cowpea, dolichos bean, French bean, peas
Leafy vegetables	*Amaranthus*, beet leaf, fenugreek, spinach, lettuce

Table 19.2 Some important seed-borne diseases of vegetables grown in different parts of the world

Vegetables	Diseases	References
Brinjal	1. Damping off	Pandey (2010)
	2. *Alternaria* leaf spot	Das and Sarma (2012)
	3. Tip over disease of eggplant	Pawar and Patel (1957)
	4. Bacterial wilt	Koike et al. (2007)
Tomato	1. *Fusarium* wilt	Rashid et al. (2016)
	2. *Phoma* blight	Rashid et al. (2016)
	3. *Pythium* damping off	Rashid et al. (2016)
	4. *Alternaria* blight	Kemmitt (2002)
	5. Bacterial wilt	Koike et al. (2007)
	6. Bacterial spot	Panagopoulos (2000)
Potato	1. Late blight of potato	Agrios (2005)
	2. *Fusarium* dry rot	Schisler et al. (2000)
	3. Bacterial soft rot	Collmer and Keen (1986)
Crucifers	1. *Alternaria* leaf spot	Kucharek (2000)
	2. *Fusarium* yellows	Ismail et al. (2012)
	3. Black rot of crucifers	Zhao and Damocone (2000)
Legumes	1. Bacterial blight	Kaiser (1981); Ghangaokar and Kshirsagar (2013)
	2. Anthracnose	Kaiser (1981); Ghangaokar and Kshirsagar (2013)
	3. *Alternaria* blight	Kaiser (1981); Ghangaokar, and Kshirsagar (2013)
	4. *Ascochyta* blight	Kaiser (1981); Ghangaokar, and Kshirsagar (2013)
	5. *Botrytis* or grey mould	Nene et al. (1996)
	6. *Fusarium* wilt	Kraft (1994)
	7. *Stemphylium* blight	Nene et al. (1996)
	8. Alfalfa mosaic	Nene et al. (1996)
	9. Bean yellow mosaic	Nene et al. (1996)
	10. Cucumber mosaic	Nazar et al. (2000)
	11. Pea seed-borne mosaic	Gao et al. (2004)
Onion and garlic	1. Damping off	Mishra et al. (2014)
	2. Purple blotch	Mishra et al. (2014)
	3. Basal rot	Barnoczkine (1986)
	4. Black mould	Mishra et al. (2014)
	5. Sour skin	Mishra et al. (2014)
	6. Bacterial brown spot	Mishra et al. (2014)
	7. Iris yellow spot	Mishra et al. (2014)
Carrot	1. *Alternaria* leaf blight	Navazio et al. (2010)
	2. Black rot	Navazio et al. (2010)
	3. Carrot bacterial blight	Navazio et al. (2010)
Ginger	1. Bacterial wilt	Jayashree et al. (2015)

(continued)

Table 19.2 (continued)

Vegetables	Diseases	References
Cucurbits	2. *Alternaria* leaf spot	Watson and Napier (2009)
	3. Anthracnose	Watson and Napier (2009)
	4. Scab of gummosis	Watson and Napier (2009)
	5. Powdery mildew	Watson and Napier (2009)
	6. Angular leaf spot	Watson and Napier (2009)
	7. Bacterial leaf spot	Watson and Napier (2009)
	8. Mosaic disease	Watson and Napier (2009)
Leafy vegetables	1. *Pythium* stem canker	Block and Van Roekel (2011)
	2. *Alternaria* leaf spot	Sikora and Kemble (2000)
	3. *Cercospora* leaf spot	Sikora and Kemble (2000)
	4. Black rot	Sikora and Kemble (2000)

Seed-borne diseases refer to plant diseases disseminated or transmitted by seed. Diseases of plants are caused primarily by three types of pathogens: bacteria, fungi and viruses. Among them, fungi constitute the largest group of pathogens, while other seed-specific diseases are caused by bacteria, viruses and few by nematodes.

19.2 Seed-Borne Diseases of Brinjal (*Solanum melongena*)

Brinjal or eggplant is an important extensively consumed nutritious vegetable crop in India and cultivated commercially throughout the tropical and subtropical regions of the world. It has been cultivated in India for the last 4000 years, although it is often thought of as a Mediterranean or mid-Eastern vegetable.

Fungal diseases play an important role in reducing the production of brinjal (Habib et al. 2007). Gangopadhyay and Kapoor (1975) revealed from an experiment that most of the fungi were located on the seed coat and tegmen which reduced the seed germination (30–82%) as compared to the control (85%). The pathogen was found located in the different component of seed as testa, tegmen and embryo in histopathology.

19.2.1 Damping Off Disease

It is a kind of seedling disease common to most of the vegetables grown for transplanting or even when direct seeded (Rout and Rath 1974; Pandey 2010). It results in poor germination and low plant stand in nursery beds and often carries the pathogen to the transplanted fields.

19.2.1.1 Aetiology

It is caused by *Pythium aphanidermatum*, a fungus belonging to *Oomycetes* group. The cell wall is made up of cellulose instead of chitin, diploid in vegetative state and contains coenocytic hyphae. They reproduce asexually with motile biflagellate

zoospores and infect the host. High soil moisture coupled with poor drainage and relatively high temperature favours rapid development of damping off.

19.2.1.2 Symptoms

Damping off of vegetables occurs in two phases:

- *Pre-emergence damping off*

In this phase of the disease, the young seedlings are killed before they emerge through the soil surface. The seeds may rot or the seedlings may get killed before the hypocotyl has broken the seed coat. The radical and plumule, when come out of the seeds, undergo complete rotting.

- *Post-emergence damping off*

This type of mortality of seedlings is very prominent. It is characterized by the drooping over of infected seedlings after they emerge from the soil. The infected tissue appears soft, stained and water soaked. As the disease advances the stem becomes constricted at the base and the plant collapse. In the fields and nurseries, the disease usually radiates from initial infection points, causing large spots or areas in which nearly all the seedlings are killed.

19.2.2 *Alternaria* Leaf Spot

19.2.2.1 Aetiology

This disease is caused by *Alternaria melongenae* which belongs to class *Deuteromycetes* and *Dematiaceae* family (Das and Sarma 2012). The conidiophores are dark, septate, simple or branched, bearing conidia at the apex. The spores are muriform, dark pigmented, ovate or obclavate, tapering towards the apex.

19.2.2.2 Symptoms

This disease causes characteristic leaf spot with concentric rings. The spots are mostly irregular 4–8 mm in diameter and coalesce to cover large areas of the leaf blade. Severely affected leaves drop off. The affected fruits form large deep-seated spots. The infected fruits turn yellow and drop off prematurely (Sokhi 1994).

19.2.3 Tip Over/*Phomopsis* Blight Disease of Eggplant

19.2.3.1 Aetiology

It is caused by *Diaporthe vexans*. Pycnidia are subepidermal, erumpent, dark, thick-walled, flattened to globose, varying in size, often 100–300 μm in diameter, with or without a beak; beak is 76 μm long. Phialides are hyaline, simple or branched, sometimes septate, 10–16 μm long, arising from the innermost layer of cells lining

the cavity. Alpha conidia are hyaline, aseptate, subcylindrical, 5–8 × 2–3 μm in size. Beta conidia are filiform, curved, hyaline, septate, 18–32 × 0.5–2.0 μm in size, and non-germinating. Hyphae are hyaline, septate, 2.5–4 μm in diameter (Singh 1987). Perithecia in culture are usually in clusters, 130–350 μm in diameter, beaked; beaks are sinuous, carbonaceous, irregular, 80–500 μm long. Asci are clavate, sessile, 24–44 × 5–12 μm and eight-spored. Ascospores are biseriate, hyaline, narrowly ellipsoid to bluntly fusoid, one-septate, constricted at the septum, 9–12 × 3–4.5 μm in size (Gratz 1942).

19.2.3.2 Symptoms

The symptoms are poor germination, seedling blight and fruit rot. Post-emergence damping off of seedlings results from infection of the stem just above the soil surface. The symptoms on leaves are more prominent during the early stages of plant growth. The lesions first are small, slightly circular with an irregular blackish margin (Pawar and Patel 1957). Irregular spots result from coalescence of the small spots. After transplanting, leaves coming in contact with the soil may become infected directly or develop leaf spot due to infection by conidia. Lesions on the petiole or the lower part of the midrib can result in death of the entire leaf. Affected leaves may drop prematurely, and the blighted areas become covered with numerous black pycnidia. On stems and branches elongated, blackish-brown lesions are formed, eventually containing pycnidia. The diseased plant bears smaller leaves and the axillary buds are often killed. When stem girdling occurs, the shoot above the infected area wilts and dries up and the plant may be toppled by the wind (Pawar and Patel 1957; Sherf and MacNab 1986). Pycnidia develop readily in lesions on young stems, but rarely on older ones (Harter 1914). On the fruits the symptoms appear first as minute sunken greyish spots with a brownish halo, which later enlarge and coalesce, producing concentric rings of yellow and brown zones. These spots increase in size and form large rotten areas on which conidiomata often develop concentrically, covering most of the rotten fruit surface. Pycnidia on fruit are larger than those on stems and leaves (Harter 1914). If the infection enters the fruits through the calyx, the whole fruit may become mummified due to dry rot (Pawar and Patel 1957). Infection in seed adversely affects the seed quality, causing seed discoloration, reduced seed weight and density, poor germinability and reduced viability (Vishunavat and Kumar 1994).

19.2.4 Bacterial Wilt

Bacterial wilt is one of the very serious diseases of more than 200 plants species including brinjal belonging to over 40 families (Panagopoulos 2000; Koike et al. 2007).

19.2.4.1 Aetiology

It is caused by *Ralstonia solanacearum*. It is a gram-negative, rod-shaped, aerobic, motile bacterium with one or four flagella.

19.2.4.2 Symptoms

The symptoms first appear as light, partial or total flaccidity of young leaves during the hot hours of the day, followed by recovery at night. Sometimes only one side of the plants may survive. Under favourable conditions, rapid and complete wilting takes place followed by collapsing and ultimately death of the plant. Internal brown discolouration of the vessels takes place on the stem near the base. When the cross sections of the stems are cut, milky ooze comes out of the cut sections within 5 min (Panagopoulos 2000; Koike et al. 2007).

19.3 Seed-Borne Diseases of Tomato (*Lycopersicon esculentum*)

Tomato is an important vegetable which has been affected by many seed-borne diseases that influence the overall health, germination and final crop stand in the field. The infected seeds fail to germinate or may transmit disease from seed to seedling and/or from seedling to growing plant (Islam and Borthakur 2012). Fungal pathogens may be both externally and/or internally seed-borne or may be associated with the seeds as contaminants (Singh and Mathur 2004). Sometimes seed quality may be lowered due to saprophytic and very weak fungal pathogens, by causing seed discolouration, thus reducing the commercial value of the seeds (Al-Askar et al. 2012). Among the seed-borne pathogens, *Fusarium oxysporum* is reported to be one of the most destructive as it can cause up to 65% reduction in germination of tomato. *Phoma destructiva* can reduce tomato germination by 58%, while *Alternaria solani* causes early blight of tomato (Mehrotra and Agarwal 2003). Other seed-borne fungi that were reported on tomato include *A. alternata, Colletotrichum gloeosporioides, Bipolaris maydis, Curvularia lunata, F. moniliforme, F. solani, F. equiseti, Cladosporium* sp., *Aspergillus clavatus*, *A. flavus, A. niger, Penicillium digitatum*, *Pythium* sp., *Verticillium* sp., *Rhizoctonia* sp., *Rhizopus arrhizus*, *R. stolonifer* and *Sclerotinia* sp. (Nishikawa et al. 2006).

19.3.1 *Fusarium* Wilt

It is one of the important seed-borne diseases of the tomato plants.

19.3.1.1 Aetiology

This is a fungal important disease, and the causal organism of this disease is *Fusarium oxysporum* f. sp. *lycopersici*.

19.3.1.2 Symptoms

The initial symptom is chlorosis of the lower leaves that often begins on one side of the plant followed by wilting of the foliage. Infected leaves later show curling, browning and drying. With the advance of the disease, the entire plant turns chlorotic, wilts, collapses and ultimately dies. Vascular brown discolouration can be

seen in infected stems and large leaf petioles. The root portions of the affected plants are stunted (Rashid et al. 2016).

19.3.2 *Phoma* Blight

Tomato plants cultivated in the Cameron Highlands Malaysia had extensive fruit, stem and leaf spot damages caused by *Phoma destructiva*, and it caused disease up to 70% in experimental plot (Rashid et al. 2016).

19.3.2.1 Aetiology

It is caused by *Phoma destructiva*.

19.3.2.2 Symptoms

Necrotic spots are formed on all aerial parts of the plant, followed by total necrosis of the plant and mummification of its fruits. Leaf lesions are initially small (1–2 mm), black on either surface, enlarging to 1–2 cm in diameter, irregular to round in shape, and slightly sunken and zonate. Stem lesions were longer but similar in appearance. Fruit lesions were sunken black spots of variable size at sites of wounds, insect punctures or stem scar cracks (Rashid et al. 2016).

19.3.3 *Pythium* Damping Off Disease

Pythium attacks tomato plant during early stages of growth, causing seed rot, pre-emergence rot of seedling, post-emergence rot of seedling and stem rot.

19.3.3.1 Aetiology

Several *Pythium* spp. (*P. aphanidermatum, P. debaryanum, P. ultimum*) are responsible for this disease.

19.3.3.2 Symptoms

- **Pre-emergence**: In this phase the seeds fail to emerge after sowing. They become soft and mushy, turn brown and decompose itself as a consequence of infection.
- **Post-emergence**: Post-emergence phase of the disease is characterized by dark coloured, water-soaked lesions on the roots which spread up the stems. Later on the seedling falls, withers and dies (Mc Carter 1991).

19.3.4 *Alternaria* Blight

Early blight is distributed worldwide and essentially occurs wherever tomatoes are grown. If left uncontrolled the disease may cause severe defoliation, resulting in reduced fruit size and number. Literature reported that crop losses due to early blight in unsprayed fields vary from 5 to 78% (Kemmitt 2002).

19.3.4.1 Aetiology

The causal organism of early blight is *Alternaria solani*, a *Deuteromycetes* fungus. The genus *Alternaria* is a large and important group of pathogenic fungi, which cause a significant number of important diseases. The fungus is readily cultured on artificial media such as V8 juice where it produces a deeply pigmented grey/black hairy colony. The mycelium is haploid and septate, becoming dark pigmented with age. Sporulation in culture can be stimulated by exposure to fluorescent light. The asexual conidia are borne singly or in a chain of two on distinct conidiophores. The beaked conidia normally possess 9–11 transverse septa. Morphological and pathogenic variability among isolates of *A. solani* has given rise to claims of the existence of races, although this remains unproven (Kemmitt 2002).

19.3.4.2 Symptoms

Initial symptoms on leaves appear as small 1–2 mm black or brown lesions. Under favourable environmental conditions the lesions enlarge and are often surrounded by a yellow halo. Lesions greater than 10 mm in diameter often have dark pigmented concentric rings. This so-called 'bulls' eye' type of lesion is highly characteristic of early blight. As lesions expand and new lesions develop entire leaves may turn chlorotic and dehisce, leading to defoliation. Lesions occurring on stems are often sunken having the typical concentric rings. On young tomato seedlings, lesions may completely restraint the stem, known as 'collar rot', symptom which may lead to death. Premature dropping of the infected fruits may also take place (Kemmitt 2002).

19.3.5 Bacterial Wilt

Bacterial wilt is a very serious bacterial disease affecting more than 200 plant species belonging to 40 families. Reports for complete loss of the diseased crops have been reported (Koike et al. 2007). It is a very serious disease problem of tomato.

19.3.5.1 Aetiology

The causative agent of the disease *Ralstonia solanacearum* is a gram positive, rod shaped, aerobic, motile bacteria with one to four motile polar flagella.

19.3.5.2 Symptoms

First symptoms of the disease appear as partial or total epinasty of young leaves during the hot hours of the day followed by recovery during night. Under conducive climate, rapid and complete wilting takes place due to this the whole plant collapses and dies. Internal brown discolouration takes place on the vessels of the stem. In cross section, oozing of tiny, white, milky drops of viscous fluid from several vascular bundles of the infected shoots takes place (Panagopoulos 2000; Koike et al. 2007).

19.3.6 Bacterial Spot

This disease is serious worldwide especially in tropical and subtropical parts of high humidity and rainfall.

19.3.6.1 Aetiology

This disease is caused by *Xanthomonas campestris* pv. *vesicatoria*. The bacterium is aerobic, gram negative and having polar flagellum.

19.3.6.2 Symptoms

Spots up to 5 mm size appear on the leaves, which are circular to irregular in shape. As the lesions enlarge, they are surrounded by yellow halo. These spots may coalesce and form large necrotic spots leading to defoliation in the severely infected seedlings. On petioles and stems, long dark streaks may develop. On fruits, the spots appear as black, raised, blisters surrounded by water-soaked areas (Panagopoulos 2000; Koike et al. 2007).

19.4 Seed-Borne Diseases of Potato (*Solanum tuberosum*)

Seed-borne diseases play a serious constraint to potato production in all parts of the world. Late blight of potato and *Fusarium* dry rot are the major seed-borne diseases of potato.

19.4.1 Late Blight of Potato

Potato late blight disease is most important disease in this crop. This is caused by fungal incitant. Potato late blight is the historical disease that triggered the Irish potato famine of the 1840s. This was the first plant disease for which a microorganism was proved to be the causal agent, leading to the birth of the plant pathology.

19.4.1.1 Aetiology

It is caused by *Phytophthora infestans*. This pathogen is classified under Oomycete which is member of the kingdom Chromista. It is distributed worldwide and severe epidemics occur in areas with frequent cool and moist weather (Agrios 2005).

19.4.1.2 Symptoms

- **On potato stems**
- It is identified by black/brown lesions on leaves and stems which are small, water-soaked areas that rapidly expand and become necrotic (Fig. 19.1). In humid conditions, this pathogen produces sporangia and sporangiophores on the infected surface areas (Schumann and D'Arcy 2000).

Fig. 19.1 Potato late blight symptoms on leaves. (Photograph courtesy of Ravindra Kumar)

- **On potato tuber**
  It is characterized by irregular shaped, slightly depressed, brown to purplish areas on the skin. Reddish to brown, dry, granular rot is found under the skin in the coloured area, extended into the tuber area of ½ inch in size (Wharton and Kirk 2007).

19.4.2 *Fusarium* Dry Rot

The disease causes devastating post-harvest losses affecting both seed potatoes and potatoes for human consumption.

19.4.2.1 Aetiology

It is association of several *Fusarium* species like *Fusarium sambucinum, F. solani, F. culmorum* and *F. avenaceum.*

19.4.2.2 Symptoms

In field, symptoms include variable seed emergence and differences in plant size. Infection begins at wound sites and once the infection occurs, it slowly enlarges in all the directions. The skin over the infected area sinks and wrinkles, sometimes in concentric rings. Internally infested areas are light brown to black as the fungus kills the cells of the tuber. Internal cavities created by dry rot infections generally contain fungal mycelium of varied colours (Schisler et al. 2000).

19.4.3 Bacterial Soft Rot

19.4.3.1 Aetiology

It is caused by *Erwinia carotovora,* gram negative, non-spore forming, facultative anaerobe, characterized by the production of large quantities of extracellular pectic enzymes that degrade cell wall to cause disease (Collmer and Keen 1986).

19.4.3.2 Symptoms

Slight vascular discolouration takes place till it completely decays on the tubers. There is occurrence of cream to tan-coloured lesions with brown margins surrounding the lesions on the tubers. The rots are characterized as soft, cheesy and granular (Tsror et al. 1999).

19.5 Seed-Borne Diseases of Cruciferous Vegetables

Cabbage, cauliflower, Brussels sprouts, broccoli, kale, kohlrabi and turnip are commonly referred to as cole crops. They are susceptible to a number of serious seed-borne diseases that must be controlled to obtain desired quality and good yields. Some of the seed-borne fungi have been found to be very destructive, causing seed rot leading to decreased seed germination (Ellis 1971; Haikal 2008).

19.5.1 *Alternaria* Leaf Spot

Alternaria leaf spot is most common fungal disease affecting different cruciferous vegetable crops.

19.5.1.1 Aetiology

It is caused by *Alternaria brassicae* and *A. brassicicola.* Morphological structure has been depicted in Table 19.3.

19.5.1.2 Symptoms

This disease is caused by *Alternaria* species, and occurs during warm and moist conditions. On seedlings, the symptoms are small, dark spots on the stem that can cause damping off or stunting of the plant. On older plants, the lower leaves are infected first with brown circular spots on the leaves. Spots have characteristic concentric rings (target spots) and sometimes yellow halo zone may surround the spots (Fig. 19.2). Infected leaves soon turn yellow and drop on the ground (Kucharek 2000).

19.5.2 *Fusarium* Yellows

19.5.2.1 Aetiology

The disease is caused by the fungus, *Fusarium oxysporum* f. sp. *conglutinans* (Ismail et al. 2012).

Table 19.3 Morphological structure of *Alternaria* spp. causing *Alternaria* leaf spot of crucifers

Fungal structure	*Alternaria brassicae*	*Alternaria brassicicola*
Mycelium	Septate, brownish grey	Septate, olive grey to greyish black
Conidiophore	Dark, septate, arise in fascicles, 14–74 × 4–8 µm	Olivaceous, septate, branched, 35–45 × 5–8 µm
Conidia	Brownish black, obclavate, muriform, produced singly or in chains	Dark, cylindrical to oblong, muriform, produced in chains of 8–10 spores
Spore body (µm)	96–114 × 17–24	45–55 × 11–16
Spore beak length (µm)	45–65	None
Transverse septation	10–11	5–8
Longitudinal septation	0–6	0–4
Infection	Penetrates leaf only through stomata	Penetrates leaf directly or through stomata

Fig. 19.2 *Alternaria* leaf spot of cauliflower. (Photograph courtesy of Ravindra Kumar)

19.5.2.2 Symptoms

The fungus usually enters the plant through young rootlets or wounds in older roots at transplanting time and then moves up the stem and throughout the plant. Symptoms include leaf yellowing, defoliation of older plants, stunting and death of seedlings. Stems are often twisted to one side. On susceptible plants, symptoms may not appear until the soil warms up, close to the time of crop maturity. It is easily confused with black rot, except discoloration inside the stem which appears more yellow-brown instead of black. Yellows are more likely to cause a curve in the midrib, resulting in a plant that is stunted to one side (Ismail et al. 2012). The field fungi cause diseases like leaf spot, seedling blight and pre- and post-emergence of seedlings leading to poor crop stand and subsequently reduced yield (Ellis 1971; Hassan 1999). The

storage fungi deteriorate the seed viability and quality, which reduce crop stand and also render the seed unfit for human and animal consumption (Otani et al. 1995).

19.5.3 Black Rot of Crucifers

It is considered to be the most serious disease of crucifers worldwide. This disease is also known as blight, black stem, black vein and stem rot (William 1980; Zhao and Damocone 2000).

19.5.3.1 Aetiology

It is caused by *Xanthomonas campestris* pv. *campestris* (Zhao and Damocone 2000).

19.5.3.2 Symptoms

Disease symptoms appear as circular to angular, interveinal necrosis with yellow halos. Lesions ranged from 2 mm to 1 cm in size. Chlorotic halos are observed on the spots. The lesion margins are usually water soaked. Later on, the spots coalesce, spread to the leaf, get necrotic and die (Zhao and Damocone 2000).

19.6 Seed-Borne Diseases of Leguminous Vegetables

A legume is a plant or fruit/seed in the family *Fabaceae*. Legumes are grown primarily for their grain seed called pulse, for livestock forage and for green manure to enhance soil fertility. It includes beans, peas, chickpeas, lentils, soybeans, peanuts, etc. Seeds are the most important input for crop production and pathogen free healthy seeds are urgently required for desired plant population (Neergaard 1969). However, the legume seeds are attacked by various seed-borne diseases resulting in enormous yield losses, and it may extent up to 50% losses (Dawson and Bateman 2001; Islam et al. 2009). Important seed-borne fungal diseases were found by blotter method by Ghangaokar and Kshirsagar (2013). A viral disease in pea, i.e., *Pea seed-borne mosaic virus*, has been reported that caused yield loss up to 100% in Sweden (Coutts et al. 2009). This disease can affect lentil, faba bean, chickpea and field pea (Ghangaokar and Kshirsagar 2013). Below are some important seed-borne diseases of leguminous vegetable crops with their causal agents and economic importance.

19.6.1 Bacterial Blight

19.6.1.1 Aetiology

Bacterial blight is a serious disease of field peas that is caused by the bacterial pathogens *Pseudomonas syringae* pv. *pisi* and *Pseudomonas syringae* pv. *syringae*. *Xanthomonas campestris* pv. *cassiae* is responsible for bacterial blight in chickpea (Nene et al. 1996).

19.6.1.2 Symptoms

The disease first appears on leaf as small, dark-green, water-soaked spots. These spots later on coalesce and turn yellowish to brown. Both pre-emergence and post-emergence damping off may occur. Heavily infected seed may be discoloured, resulting in poor seed quality. Severe epidemics can occur which can lead to crop failure. The pathogens are seed-borne. Infected and contaminated seeds are the most important sources of inoculum for disease epidemics in the field. The pathogens also survive on stubble and in the soil (Kaiser 1981; Ghangaokar and Kshirsagar 2013).

19.6.2 Anthracnose Disease

19.6.2.1 Aetiology

Anthracnose of lentil and chickpea is caused by the fungus *Colletotrichum truncatum*. The fungus causing anthracnose in field pea is *C. pisi*. Anthracnose of lentil is an economically important disease in all parts of the world, where it is grown. Anthracnose is an emerging problem of pea (Kaiser 1981).

19.6.2.2 Symptoms

The disease symptoms include tan colour lesions which can lead to defoliation and girdling, causing plants to wilt and lodge. Seeds from infected plants may be discoloured and shriveled, resulting in significant losses. In pea, anthracnose reduces productivity and germination ability of seeds.

19.6.3 *Alternaria* Blight

19.6.3.1 Aetiology

Alternaria blight is caused by a fungus, *Alternaria alternata*. It infects chickpea, lentil and field pea.

19.6.3.2 Symptoms

The pathogen is seed-borne and it can be spread by planting infected seeds (Kaiser 1981). It causes lesions on leaf margins and tips, petioles, flowers and pods, similar to those caused by *Ascochyta*. Affected leaflets drop off the plant. *Alternaria* can cause germination loss, and severe losses later in the growing season.

19.6.4 *Ascochyta* Blight

19.6.4.1 Aetiology

Ascochyta blight is a disease complex caused by *Ascochyta* species (fungi), and other pathogens in field pea (Tran et al. 2014). The species of *Ascochyta* are host-specific. *Ascochyta rabiei* only infects chickpea and *Ascochyta lentis* only infects lentil. In field pea, *Ascochyta* blight is caused by a complex of three pathogens, viz.

Ascochyta pisi, *Mycosphaerella pinodes* (asexual stage: *Ascochyta pinodes*) and *Phoma pinodella*.

19.6.4.2 Symptoms

The pathogens cause lesions (disease spots) on every part of the pulse crop they infect and can easily spread in the field. These lesions coalesce to form blight. The disease can reduce seed yield and seed quality. This pathogen can be seed-borne at high levels (Kaiser 1981).

19.6.5 *Botrytis* or Grey Mould

19.6.5.1 Aetiology

Botrytis grey mould is caused by *Botrytis cinerea* in chickpea and lentil (Nene et al. 1996).

19.6.5.2 Symptoms

The pathogen causes symptoms, which at onset appears as water-soaked lesions on stems, branches, leaves, flowers and pods, then progress to grey/brown lesions, and are often covered with a grey mass of fungal hyphae and spores. The pathogen prefers blossoms and pods but can also attack other aerial parts of the plant. The disease causes flowers to drop, resulting in significant seed yield losses. Seedling soft-rot of chickpea can arise from infected seeds. The fungus can survive on infected seed for up to five years.

19.6.6 *Fusarium* Wilt

This disease, caused by *Fusarium oxysporum*, was responsible for the decline of the pea industry in many regions of the United States including Montana's Gallatin valley during the middle 1900s.

19.6.6.1 Aetiology

Fusarium wilt is caused by subspecies of *Fusarium oxysporum* specific to crop. For example, *F. oxysporum* f. sp. *pisi* infects pea, *F. oxysporum* f. sp. *lentis* infects lentil, and *F. oxysporum* f. sp. *ciceris* infects chickpea (Kraft 1994).

19.6.6.2 Symptoms

Symptoms include chlorotic leaflets, which curl downwards and become flaccid. The plant wilts slowly and turns yellowish brown in colour. The above-ground vascular system turns light yellow to brick coloured. The lower portion of the stem becomes larger than normal size (Kraft 1994).

19.6.7 *Stemphylium* Blight

19.6.7.1 Aetiology

Stemphylium blight (chickpea, lentil) is caused by *Stemphylium botryosum*. It is a disease of increasing importance of lentil in Canada. The fungus also infects field pea.

19.6.7.2 Symptoms

It causes leaf spots which coalesce causing defoliation of the plant. The pathogen can be seed-borne, and infected seeds have low germination rate (Nene et al. 1996).

19.6.8 Alfalfa Mosaic Disease

19.6.8.1 Aetiology

Alfalfa mosaic virus (AMV) is the causal organism of this disease and survives in infected seed or plant hosts (Nene et al. 1996).

19.6.8.2 Symptoms

It typically induces a bright yellow mottle or mosaic symptom. In field pea and chickpea, it causes chlorosis and necrosis of the new shoots. Pods may be malformed and fail to develop peas. Lentils may develop necrotic tip growth, twisting and deformation of leaves and stunting when infected with the virus. The virus is seed-borne.

19.6.9 Bean Yellow Mosaic Disease

19.6.9.1 Aetiology

Bean yellow mosaic virus (BYMV) is the causal organism (Nene et al. 1996) and is distributed worldwide with a wide host range which includes the temperate pulses.

19.6.9.2 Symptoms

The virus spreads by a number of aphid species which are non-persistent as well as being seed and mechanically transmitted. Symptoms on field peas are variable. The virus may be symptomless or may induce bright mosaic, mottling of leaves and clearance of veins. Necrosis may occur on tips, in stems and veins. Desi and Kabuli chickpeas develop apical necrosis, reddening or yellowing, plant stunting and premature senescence. Lentils develop mild mosaic, light green or yellow leaves. A reduction in leaf size and stunting may occur. Infected plants produce very little seed.

19.6.10 Cucumber Mosaic Disease

19.6.10.1 Aetiology

Cucumber mosaic virus (CMV) causes severe disease in lentils, chickpeas and lupins. It also infects field pea.

19.6.10.2 Symptoms

In areas where large aphid populations occur, crop losses can be high due to reduced herbage production and grain yield. The virus is transmitted by a number of aphid species, and it is seed-transmitted in many pulse species. CMV has the widest host range of any known plant virus and is distributed worldwide. The host range includes a large number of agricultural and horticultural crops including temperate pulses (Nazar et al. 2000).

19.6.11 Pea Seed-Borne Mosaic Disease

19.6.11.1 Aetiology

Pea seed-borne mosaic virus (PSbMV) is distributed worldwide (Gao et al. 2004).

19.6.11.2 Symptoms

The virus may be symptomless, or show mild mosaic symptoms on field peas and other legumes. Early infections may cause considerable yield losses. PSbMV also affects seed quality by causing brown ring patterns and spots on the seeds of field pea, faba bean, lentil, chickpea, lathyrus and other legumes. The virus is believed to have spread worldwide through the exchange of infected seed (Nazar et al. 2000).

19.6.12 Pea Enation Mosaic Disease

19.6.12.1 Aetiology

Pea enation mosaic disease is caused by two symbiotic viruses: *Pea enation mosaic virus*-1 (PEMV-1; an Enamovirus) and *Pea enation mosaic virus*-2 (PEMV-2; an Umbravirus).

19.6.12.2 Symptoms

Symptoms in peas typically include, in sequence, vein-clearing, mosaics, plant growth malformations, stunting and enations (outgrowths) on the veins of the underside of the leaves. Infected pods are usually malformed, look warty, and contain few, if they have seeds. Symptoms in lentils include stunting, rolling of leaves, mottling, and tip wilting or necrosis. They can also infect chickpea. PEMV is seed-borne and could be seed transmissible (Nazar et al. 2000).

19.7 Seed-Borne Diseases of Onion and Garlic

Onion and garlic are among the most important commercial vegetable crops grown all over world and is being used in India as vegetables, spices or medicines. India ranks second next to China in area and production both for onion and garlic. However, these two crops are being attacked by several seed-borne diseases (FAOSTAT 2010).

19.7.1 Damping Off Disease

It is an important fungal disease of onion and garlic, causing up to 75% yield loss in nursery stage of both the crops.

19.7.1.1 Aetiology

It is a complex disease caused by a group of pathogens comprising of *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Fusarium* sp.

19.7.1.2 Symptoms

There are two types of symptoms. The pre-emergence damping off results in seed and seedling rot before emerging out of the soil. In the post-emergence damping off the pathogen attacks the collar region of the seedlings on the surface of the soil, the collar region rots, and seedling collapse and die (Mishra et al. 2014).

19.7.2 Purple Blotch Disease

It is one of the most important diseases in all onion and garlic growing regions in the world (Mishra et al. 2014).

19.7.2.1 Aetiology

It is caused by *Alternaria porri.*

19.7.2.2 Symptoms

Purple blotch appears as small, whitish sunken lesions. These spots later turn brown, increase in size, enlarge and become zoned, somewhat sunken and more or less purplish. The lesions are visible on leaves, flower stalk and floral parts of the onion and garlic plants. The borders of the lesions are reddish in colour and surrounded by yellow halo (Fig. 19.3). Under favourable conditions, girdling of the leaves and stems occur which may turn yellow, die back, collapse and die.

Fig. 19.3 Purple blotch disease of onion. (Photograph courtesy of Ravindra Kumar)

19.7.3 Basal Rot or Bottom Rot

This disease can cause up to 90% loss (Barnoczkine 1986).

19.7.3.1 Aetiology

This disease is caused by a fungus, *Fusarium oxysporum* f. sp. *cepae*

19.7.3.2 Symptoms

At first yellowing of the leaves takes place with stunted growth of the plants; later on, leaves dry from tip downwards. In early stage of infection, the roots of the plants become pink in colour and rot later. In due course of time, the bulb decays from the lower ends and ultimately the whole plant dies (Mishra et al. 2014).

19.7.4 Black Mould

It is an important post-harvest disease under hot and humid climate and very common in place like India.

19.7.4.1 Aetiology

It is caused by *Aspergillus niger.*

19.7.4.2 Symptoms

It is characterized by the black powdery mass of spores that appears on the exterior parts of the scales and thereby reduces the market value of the bulbs (Mishra et al. 2014).

19.7.5 Sour Skin

It is primarily a disease of onion, caused by bacterial incitant. This disease can cause loss in individual field ranging from 5 to 50% (Mishra et al. 2014).

19.7.5.1 Aetiology

It is caused by *Pseudomonas cepacia*. It is an obligate aerobic, rod shaped bacterium, 1.6–3.2 × 0.8–1.0 μm in size. They occur singly or in pairs, motile, having polar flagella.

19.7.5.2 Symptoms

Slimy, pale yellow to light brown decay and breakdown of one or a few inner bulb scales are the initial disease symptoms (Mishra et al. 2014). Adjacent outer scales and the centre of the bulb may remain firm. Externally the bulb looks sound but neck region often soften after the leaves have collapsed.

19.7.6 Bacterial Brown Spot

19.7.6.1 Aetiology

It is caused by *Pseudomonas aeruginosa.*

19.7.6.2 Symptoms

Infection occurs during storage through wounds. The rot begins at the neck of the bulbs which later gives foul smell through the neck when squeezed (Mishra et al. 2014).

19.7.7 Iris Yellow Spot

19.7.7.1 Aetiology

It is caused by *Iris yellow spot virus* which is a Gemini virus.

19.7.7.2 Symptoms

It has characteristic yellow to straw coloured lesions. Lesions may be more or less round with or without a necrotic centre, or it may be diamond shaped. Lesions will appear both in seed stalks and leaves. Seed stalks may swell at the point of infection (Mishra et al. 2014).

19.8 Seed-Borne Diseases of Carrot (*Daucus carota*)

Carrot is a widely grown root vegetable of the Apiaceae family. It is an important source of *alpha*- and *beta*-carotene, precursors of vitamin A in human nutrition worldwide. Carrot is grown as an annual for its fleshy root, but is a true biennial

requiring two years for flowering and seed production. Carrot is attacked by several seed-borne diseases. The seed-borne diseases in which seed is the primary mode of transmission are of high concern as they can significantly affect seed quality and possess the potential for infection of the next crop. The following diseases are seed transmitted and commonly tested for in commercial seed production, *Alternaria* leaf blight (*Alternaria dauci*), black rot (*Alternaria radicina*) and carrot bacterial blight (*Xanthomonas campestris* pv. *carotae*).

19.8.1 *Alternaria* Leaf Blight

Alternaria leaf blight is common in seed crops grown across the world. Pryor et al. (2000) reported disease incidence of 63–99% on mature plants in a survey of eight fields in the Cuyama Valley of California. This fungal foliar disease occurs in all carrot growing regions and is transmitted easily via the seed. *Alternaria dauci* survives in the overwintered crop, crop debris in the soil and on the seed (Navazio et al. 2010).

19.8.1.1 Aetiology

It is caused by *Alternaria dauci.* Conidia are typically borne singly, but occasionally sturdy, terminal secondary conidiophores bearing a secondary spore are produced. Conidia are medium to dark olive-brown, long ellipsoid to obclavate, 60–100 × 15–25 µm (spore body), with 7–11 trans-septa and 1–3 longisepta in fewer than half of the transverse segments. Mature conidia are rostrate with a terminal filamentous beak 80–250 × 5 µm tapering distally at top occasionally with a single lateral branch up to 100 µm in length (Neergaard 1945).

19.8.1.2 Symptoms

It appears first on older leaves 8–10 days after infection as greenish brown, irregularly shaped leaf spots usually by mid-season. Petioles can become infected under increasing pressure and spots may grow and coalesce causing petiole girdling and leaf die back. Under severe pressure the most susceptible varieties can lose a considerable amount of foliage by the end of the season, resulting in depressed yields (Navazio et al. 2010).

19.8.2 Black Rot

19.8.2.1 Aetiology

It is caused by *Alternaria radicina.*

19.8.2.2 Symptoms

Black rot symptoms often first appear in the foliage of carrots as dark brown lesions on the lower portion of the petioles (Navazio et al. 2010). From there it commonly

attacks the leaf-stem base at the crown of the root, resulting in dark cankers on the root with defined margins between healthy and infected tissues. Root symptoms may not occur until late in the season, but black rot can damage crowns to the point where the foliage dies back and the apical growing point is damaged or dies. This can occur throughout the winter and can destroy roots that are overwintered for seed production, either in the field or in storage. Infected plants with viable growing points may survive into the second season but may be stunted and result in reduced seed yields. Seed harvested from black rot infested fields may have infected seed. It commonly overwinters in debris in the soil.

19.8.3 Carrot Bacterial Blight

19.8.3.1 Aetiology

It is caused by *Xanthomonas carotae* pv. *carotae.*

19.8.3.2 Symptoms

Foliar symptoms start as small irregularly shaped, yellow, water-soaked lesions. These spots become brown and necrotic over the time with a yellow halo at their margins. As infections of *X. carotae* progresses dark elongated lesions may be found on the petioles. This pathogen is problematic for the seed grower as flower stems of bolting carrot plants may be dwarfed, chlorotic and become brittle. Umbels are often reduced in size and flowers may not fully develop. As lesions develop on the flower stalk, they will ooze a bacterial exudate and be sticky to the touch. When the disease becomes severe the growing points of new flower shoots will appear to melt in the bacterial exudate as they emerge. Seed is readily contaminated, and the disease can be transmitted via the seed (Navazio et al. 2010).

19.8.4 Beet Leafhopper-Transmitted Virescence

The only known vector for BLTV is the beet leafhopper (*Circulifer tenellus*), although this is still being investigated. The impact of this disease on carrot seed crops seems to be greater than on carrot vegetable production (Navazio et al. 2010).

19.8.4.1 Aetiology

This is caused by BLTV.

19.8.4.2 Symptoms

Symptoms include chlorotic foliage with older leaves that turn reddish purple and floral parts that are distorted, malformed and can be sterile. Flowers will often bolt early and develop a green caste as the petals will turn green due to a loss of pigment in petal cells. Seed yields can suffer severe losses (Navazio et al. 2010).

19.9 Seed-Borne Diseases of Ginger (*Zingiber officinale*)

Ginger (*Zingiber officinale* Rosc.) of *Zingiberaceae* family is an herbaceous perennial, the rhizomes of which are used as a spice. India is a leading producer of ginger in the world and during 2012–2013, the country produced 7.45 lakh tonnes of the spice from an area of 1.58 lakh hectares (Jayashree et al. 2015). Ginger is cultivated in most of the states in India. However, states namely Karnataka, Orissa, Assam, Meghalaya, Arunachal Pradesh and Gujarat together contribute 65% to the country's total production. One of the important seed-borne diseases of ginger is the bacterial wilt.

19.9.1 Bacterial Wilt

19.9.1.1 Aetiology

It is caused by *Ralstonia solanacearum*, biovar-3.

19.9.1.2 Symptoms

Water-soaked spots appear at the collar region of the pseudostem and progress upwards and downwards. The first conspicuous symptom is mild drooping and curling of leaf margins of the lower leaves which spread upwards. In the advanced stage, the plants exhibit severe yellowing and wilting symptoms. The vascular tissues of the affected pseudostems show dark streaks. The affected pseudostem and rhizome when pressed gently extrudes milky ooze from the vascular strands. Ultimately rhizomes rot, emitting a foul smell (Jayashree et al. 2015).

19.10 Seed-Borne Diseases of Cucurbits

Cucurbits include watermelons, cantaloupes, cucumbers, zucchini, pumpkins, squash, bitter melons, gourds and hairy melons. They are grown in almost all the parts of the world. Cucurbits are warm weather crops which are sown, grown and harvested over spring, summer and autumn. This crop is subjected to several diseases and few of them being seed-borne diseases.

19.10.1 *Alternaria* Leaf Spot

19.10.1.1 Aetiology

This disease is caused by two fungi *Alternaria cucumerina* and *Alternaria alternata*.

19.10.1.2 Symptoms

Small spots develop on the upper surface of leaves which may develop into larger coalescing lesions under favourable conditions. These lesions might have concentric rings (Watson and Napier 2009).

19.10.2 Anthracnose Disease

19.10.2.1 Aetiology

This is caused by fungus *Colletotrichum orbiculare.*

19.10.2.2 Symptoms

Brown to black spots develop on leaves; long dark spots develop on stems and round sunken spots develop on fruit. Fruit symptoms might develop in transit (Watson and Napier 2009).

19.10.3 Scab or Gummosis

19.10.3.1 Aetiology

It is caused by fungus *Cladosporium cucumerinum.*

19.10.3.2 Symptoms

It can affect leaves, petioles, stems and fruits. Water-soaked spots occur on leaves and runners (Watson and Napier 2009). These spots eventually turn grey to white. The centre of the spots could then drop out to give a 'shot-holed' appearance. Lesions on fruit are often confused with anthracnose. These spots are 3–4 mm in diameter and might ooze a gummy substance. The spots could then be invaded by secondary rotting bacteria which cause the spots to smell.

19.10.4 Powdery Mildew

19.10.4.1 Aetiology

It is caused by fungus, *Podosphaera xanthii.*

19.10.4.2 Symptoms

White powdery spots develop on leaves. Symptoms usually develop first on the underside of leaves and later these cover both sides. Leaves gradually turn yellow to papery brown and eventually die.

19.10.5 Angular Leaf Spot

19.10.5.1 Aetiology
This disease is caused by a bacterium, *Pseudomonas syringae* pv. *lachrymans.*

19.10.5.2 Symptoms
On leaves the disease first appears as small, water-soaked spots which enlarge to about 3 mm in diameter. The spots become tan on the upper surface and gummy or shiny on the lower surface, due to bacterial ooze which dries out and turns white. Round lesions occur on fruit. Mainly it occurs in cucumbers, but also found on rock melon, honeydew melon, watermelon and squash (Watson and Napier 2009).

19.10.6 Bacterial Leaf Spot

19.10.6.1 Aetiology
It is caused by bacterium, *Xanthomonas campestris* pv. *cucurbitae.*

19.10.6.2 Symptoms
Spots first appear on squash and pumpkin leaves as small, water-soaked or greasy areas on the underside of leaves, and as indefinite yellow areas on the upper side of leaves. In about 5 days the spots become round to angular with thin, brown, translucent centres and a wide, yellow halo. These spots enlarge up to about 7 mm in diameter. Occasionally young stems and petioles are attacked. Young fruit may also be affected. Fruit may produce light brown ooze from small, water-soaked areas, which can extend into the seed cavity, causing seed infection (Watson and Napier 2009).

19.10.7 Mosaic Disease

19.10.7.1 Aetiology
It is caused by *Watermelon mosaic virus* (Types 1 and 2), *Papaya ring spot virus* and *Zucchini yellow mosaic virus*.

19.10.7.2 Symptoms
There is light and dark green mottling of the leaves. Distortion of leaves and stunting of the plant might occur. Marrow and summer squash fruit might show sunken concentric circles or a raised marbled pattern. *Papaya ring spot virus* may cause lumpy distorted fruit on zucchini. Viruses may also affect fruit set. All commercially grown cucurbits are susceptible. Pumpkin, squash, rock melon and zucchini crops are most commonly affected (Watson and Napier 2009).

19.11 Seed-Borne Diseases of Leafy Vegetables

It is comprised of leafy greens, vegetables or green plant leaves which are consumed as vegetables accompanied by tender petioles and shoots. They are kale, spinach, amaranthus, green mustard, lettuce, etc.

19.11.1 *Pythium* Stem Canker

19.11.1.1 Aetiology
It is caused by *Pythium aphanidermatum* (Block and Van Roekel 2011).

19.11.1.2 Symptoms
An unusual disease because it forms cankers (dead sunken tissue) above the soil line (Block and Van Roekel 2011).

19.11.2 *Phomopsis* Leaf Blight and Stem Canker

19.11.2.1 Aetiology
It is caused by the fungus *Phomopsis amaranthicola*.

19.11.2.2 Symptoms
It causes rapid defoliation and plant death of susceptible species (Block and Van Roekel 2011). Particularly devastating to *Amaranthus tricolor*.

19.11.3 *Alternaria* Leaf Spot

It is a common problem on collards, kale, mustards and turnips. The disease can reduce plant vigour resulting in an unmarketable crop where the leaf is the edible portion of the plant.

19.11.3.1 Aetiology
It is caused by several species of fungus *Alternaria*, viz., *A. brassicae*, *A. brassicicola* and *Alternaria raphani* (Sikora and Kemble 2000).

19.11.3.2 Symptoms
The initial symptoms are small pinpoint-size dark circular spots on the surface of older leaves. With age, spots enlarge from 2 to 3 inches in diameter and are black, brown or tan coloured. Concentric rings may develop within the lesion, and a yellow halo may be present around the lesion edge. The centre of a spot may eventually

drop out, producing a shot-hole appearance or under wet conditions, become covered with a greenish-black or brown velvety fungal mould. If left uncontrolled, the disease can defoliate the plant. Roots of turnips can become infected, especially after harvest. Wounding of roots promotes infection. Spots on roots are circular, zonate, with various shades of brown to black colour. These firm textured root lesions may extend into the centre of the root.

19.11.4 *Cercospora* Leaf Spot, White Spot and Anthracnose

These three fungal leaf diseases are common on greens in Alabama and can be easily confused for each other. Development of *Cercospora* leaf spot (*Cercospora brassicicola*), white spot (*Pseudocercosporella capsellae*) and Anthracnose (*Colletotrichum higginsianum*) is favoured by wet conditions (Sikora and Kemble 2000).

19.11.4.1 Symptoms

Cercospora leaf spot causes leaf lesions that vary in colour from pale green to grey to white and generally have a brown border. Lesions may be circular or angular in appearance. Severely infected plants may become defoliated. The disease is also known as frogeye leaf spot. White spot causes circular spots with grey, brown, or nearly paper-white centres with slightly darkened margins. Spots are formed on the cotyledons, leaves and petioles. Infected foliage may turn yellow and drop prematurely. Seedlings can be killed when the disease is severe. Anthracnose can be a serious problem on collards, kale, mustards and turnips. Anthracnose leaf spots are small, pale grey to straw coloured, dry and circular. Spots can also appear on stalks and are elongated, sunken and grey to brown and have a black border. The fungus can also attack turnip roots, causing small dry sunken areas that become tan to grey in colour, and spots are deeper in depth which enlarges in due course of time. The disease often predisposes infected roots to bacterial soft rot (Sikora and Kemble 2000).

19.11.5 Black Rot

It is a problem on crucifers, including collards, kale, mustards and turnips.

19.11.5.1 Aetiology

It is caused by the bacterium *Xanthomonas campestris*.

19.11.5.2 Symptoms

Seedling infection first appears as blackening along the margins of the cotyledons. Cotyledons shrivel and drop off. Infected seedlings may be stunted and yellow and may eventually wilt and die. Seedling infection can be difficult to diagnose since only a few plants in a lot may be infected. The disease is easily recognized on most crucifers by the presence of yellow, V-shaped or U-shaped areas extending inwards from the margin of the leaf. The bacteria usually infect the plant through hydathodes

(water pores) at leaf margins. As the disease progresses, the yellow lesions turn brown and the tissues die. Veins darken and the midrib of leaves turn black within the affected leaf area. This vein discolouration progresses towards the base of the leaf as the bacteria spread through the leaf veins. Eventually, the bacteria spread into the main stem. When infected stems are cut in cross section, a black vascular ring may be evident where bacteria have moved into the water-conducting vessels. The vascular discolouration extends from the stem to the upper leaves and down into the roots. In later stages of the disease, all central tissues of the main stem turn black (Sikora and Kemble 2000).

19.12 Management Practices

19.12.1 Cultural Practices

Selection of sowing dates for favourable conditions for the crops but unfavourable conditions for the plant pathogens. Early sowing of well-stored clean certified seed after deep ploughing, clean cultivation and timely weeding may reduce the disease pressure (Meena et al. 2002). Crop rotation with non-host, resistant or antagonistic crops and basal soil application of potash (K) has been found to check disease in crops (Sharma and Kolte 1994). Care must be taken to avoid injury during transplanting. Some fungi, bacteria and viruses can enter through injury (*Septoria apiicola*, *Xanthomonas campestris* pv. *campestris*, *Tobacco mosaic virus* etc.). After harvest the seeds or crop debris should be burnt or incorporated into the soil and the alternate hosts, volunteers, weeds, etc. should be destroyed. Overhead irrigation practices should be avoided. Proper spacing/plant population should be maintained to avoid favourable condition (microclimate) for pathogens.

19.12.2 Physical Practices

Hot water treatment of seed at 50 °C for 30 min (*Xanthomonas campestris* pv. *campestris*).

19.12.3 Biological Practices

Phytoextracts contain natural antimicrobial properties which restricts the microbial growth (Begum et al. 2010). Some essential oil extracts from tea tree, clove, peppermint, rosemary, laurel, oregano and thyme oils, etc. have been reported to have antifungal activity against pathogens like *Ascochyta* spp., which are responsible for *Ascochyta* blight on *Fabaceae*, and *Alternaria* spp., which affect carrot seeds. Thyme oil contains thymol, and other antimicrobial compounds have been reported to be active against seed-borne diseases (Van der Wolf et al. 2008).

Biological control of plant pathogens is one of the eco-friendly, alternative and realistic approach for managing pathogens. It is reported that *Trichoderma* spp. is quite effective as seed treatments against seed-borne pathogens (Harman 2006; Verma et al. 2007; Ha 2010). *Pseudomonas fluorescens, Bacillus* spp. and plant growth-promoting rhizobacteria (PGPR) are also effective against the seed-borne diseases (Gutiérrez-Mañero et al. 2001; Whipps 2001; Idris et al. 2007; Choudhary and Johri 2008; Richardson et al. 2009; Kumar et al. 2011).

19.13 Conclusion

With an augment of never ending human populations, it has become difficult to cope up with present production and productivity of vegetables globally. Besides due to the climate change and global warming, there is an increase in intensity of various seed-borne diseases caused by many fungi, bacteria and viruses. Development of disease due to seed-borne inoculum is result of the consequences of the interactions between the aggressive pathogen, vulnerable host and the favourable environment. Thus, it is inevitable to identify and recognize the causative agents of various seed-borne diseases causing economic threat in the important vegetables and thereby formulate economic, easily accessible and effective control measures which include cultural, physical, biological and chemical methods.

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Major Seed-Borne Diseases of Important Forage and Fibre Crops: Symptomatology, Aetiology and Their Economic Importance

20

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Abstract

Since long, natural fibres have been at the crux for the textile industry as a base material for making cloth, paper and building materials. As compared to synthetic fibres gaining importance today, natural fibres are a completely renewable resource and, from an environmental point of view, provide many benefits. Similarly, agriculture in accordance with animal husbandry is closely associated with farming community in a cultural and economical ways as livestock rearing and farming is an integral part of rural livelihood. For this forage crops are playing an important role in sustainable livelihood in agriculture. Occurrence of the diseases affects the availability of quality fibre and fodder. Hence, integrated disease management is the best strategy to minimize the fibre and forage crop losses caused by several diseases. These practices are eco-friendly and economical. Sowing of disease-free certified seed is very effective way to reduce disease infection at early stages and possible losses. Also, there is need for precise, reliable, rapid and proper diagnostics of seed-borne diseases for their effective management.

20.1 Introduction

Plant diseases associated with biotic stresses are primarily due to three types of causal agents, viz. fungi, bacteria and viruses. Of these fungi consist of the largest group of pathogens. The diseases caused by biotic entities transmitted by seed are

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referred as seed-borne disease. Seed deterioration refers to the breakdown of the cotyledon and embryo tissue within the seed (Kirkpatrick and Rothrock 2001). Use of infected seed/planting materials (rhizomes, cuttings, plantlets, seedlings) may result in an initiation, establishment and widespread distribution of disease-causing agents inside the plant system and an increased quantum of infectious propagule from which the disease can spread. Since there is a high rate of seed-to-seedling transmission of disease, even a small percentage of infected seed can result in significant seedling infection in the field.

The chances of infection due to seed-borne disease depend mainly on crop species, type of disease, geographic location of the crop and conducive environment. Mostly, diseases associated with seed may also be air-borne or soil-borne, and the infection depends on the varietal resistance, crop management practices and also the presence of seed-borne inoculum. In this chapter we will discuss about seed-borne diseases of important fibre and forage crops, their symptomatology and their economic importance.

20.2 Major Seed-Borne Diseases in Important Fibre Crops

Fibre crops are plants that are intentionally or traditionally grown for the production of fibre or fibrous materials with varied uses to make different things like paper, cloth or rope. Fibres are made up of sclerenchymatous cells which are the main components of plant skeleton and are mainly concerned with vascular tissues. Any part of the plant such as the roots, stems, leaves, fruits and seeds can have fibres, but they differ in characteristics such as texture, length, colour, strength and chemical composition. There are several ways of classifying fibres derived from fibre crops. As to location, fibres can be either xylary fibres if found in the xylem or extra-xylary fibres if they occur elsewhere. Extra-xylary fibres are important in the manufacture of fabrics because they are usually longer than xylary fibres (Moore et al. 2003). While on the basis of origin, fibres can be further classified into bast fibres, wood fibres, leaf fibres and surface fibres. Bast fibres are obtained from the outer parts of the stem, while surface fibres can be obtained from the hairlike projections as in the seeds of cotton. But Hill (1972) advised that for clarity on the morphological origin of bast fibres, the following descriptive terms can be used: *cortical fibres, pericyclic fibres* and *phloem fibres*. The major fibre crops are also differentiated according to the main usage of their fibres although any one fibre can have multiple uses. According to Hill (1972) based on utility, fibres can be classified as textile fibres, brush fibres, plaiting and rough weaving fibres, filling fibres, natural fabrics, paper-making fibres. Considering this above classification, we are categorising important natural fibre crops as given in Table 20.1.

Table 20.1 List of important natural fibre and their classification

Common name	Botanical name	Family
a) Surface fibre		
Cotton	*Gossypium hirsutum, Gossypium arboreum, Gossypium herbaceum, Gossypium barbadence*	Malvaceae
b) Soft or bast fibres		
Flax	*Linum usitatissimum* L.	Linaceae
Jute	*Corchorus capsularis* L and *C. olitorius* L	Tiliaceae
Kenaf	*Hibiscus cannabinus* L	Malvaceae
Sunn hemp	*Crotalaria juncea* L	Fabaceae
Ramie	*Boehmeria nivea* L. Gaud.	Urticaceae
Hemp	*Cannabis sativa* Linn.	Cannabaceae
Roselle	*Hibiscus sabdariffa* L.	Malvaceae
c) Hard or structural fibres		
Sisal hemp	*Agave sisalana* Perr.	Amaryllidaceae
Pineapple	*Ananas comosus*	Bromeliaceae
Coconut	*Cocos nucifera*	Palmae/ Arecaceae

20.2.1 Cotton (*Gossypium* sp.)

Cotton is the most important fibre crop of the entire world. It provides the basic raw material (cotton fibre) to cotton textile industry and plays a major role in agricultural and industrial economy of the country. Several soil-borne and seed-borne fungi can infect cotton seedlings individually or in association with disease complex (Hillocks and Waller 1997). A wide range of fungi may participate in the process of seed deterioration, but a few of these fungi causes pre- and post-emergence damping-off in seedlings. Seed-borne fungi may affect uniform emergence, vigorous and uniform stand of healthy seedling. If the fungi have been virulent in seedling, germination can be delayed or may not occur (Smith 1950; Arndt 1953; Roncadori et al. 1971; Lima et al. 1988). Among the cotton diseases, the root rot and bacterial blight (BLB) are most important diseases caused by seed-borne pathogens.

20.2.1.1 Root Rot Disease

(a) *Economic Importance*

Root rot caused by *Rhizoctonia solani* Kuhn and *R. bataticola* (Taub) is one of the most serious diseases of cotton particularly in the irrigated Northern cotton zone in 14 lakh hectares of area, consisting of Haryana, Punjab and Rajasthan states of India. The disease incidence is observed in June and is characterized by sudden and complete wilting of the plants (Monga and Raj 1996a).

Fig. 20.1 Root rot disease of cotton

(b) *Symptomatology*

There is sudden wilting and drooping of plants from top to bottom down. Affected plants can be easily pulled out from the ground and bark of roots breaks into shreds and gives yellowish appearance. Symptoms can be differentiated on the basis of discolouration and wetness of the infectious root. In case of *R. solani* infection, the root becomes brown and wet while black and dry root is the typical sign of *R. bataticola* infection. Whereas, in case of *Sclerotium rolfsii* infection, the mycelial growth of the fungus is observed on the roots and collar region of the plant and produce small spherical sclerotial bodies leading to rotting of roots and drying of seedlings (Fig. 20.1).

(c) *Aetiology*

The root rot disease is caused by *Rhizoctonia solani*, *R. bataticola* and *Sclerotium rolfsii. R. solani* and *R. bataticola* are the sclerotial stages of fungi of class Mycelia Sterilia. The sclerotia of *R. bataticola* are irregular in shape, black and having an average dimension of 105 ± 2 μ while those of *R. solani* are dark brown in colour with irregular size and shape. Pycnidial stage of *R. bataticola* stage is known as *Macrophomina phaseolina* (Maubl.) Ashby, it may be seed or soil-borne. The *Thanatephorus cucumeris* (Frank) Donk which produces basidia and basidiospores is the perfect stage of *R. solani.* The disease is first observed in June and becomes severe during the month of July. High soil moisture in combination with high temperature is favourable for infection (Monga and Raj 1996a, b).

(d) *Disease Management*

Deep ploughing during land preparation and exposure to solar radiation help to minimize the disease incidence. Removal and destruction of infected plant debris

should be followed. Seed treatment with *Trichoderma harzianum* or *T. viride* @ 4.0 g/Kg of seed and soil drenching of carbendazim @ 2 g/l water is recommended for disease management (Monga and Raj 1997).

20.2.1.2 *Fusarium* Wilt

(a) *Distribution and Economic Importance*

In the main cotton-producing countries, significant crop losses have been reported due to *F. oxysporum* f. sp. *vasinfectum* causing wilt. Where the disease is endemic, the losses are much greater in localized areas. *Fusarium* wilt of cotton is a harmful disease, and in infected susceptible varieties, yield losses up to 45–50% are reported.

(b) *Aetiology*

Causal Organism: *Fusarium oxysporum* f. sp. *vasinfectum*

Disease appears in 30–120 days after sowing in heavy black alkaline soils and specifically on desi (*arboreum*) and asiatic (*herbaceum*) cottons. The causative fungus has only anamorphous stage in its life cycle. The pathogen consists of morphological structures as colourless or colourful multicellular mycelium which forms macro and microconidia, and chlamydospores in soil. The primary infection sources of the fungus are infected cotton seeds and crop debris. Infection of cotton plant takes place by pathogen occurring in soil through plant roots (Hillocks and Kibani 2002). On affected cotton, at humidification, the fungus forms typical light pink mycelium with colourless multicellular curved conidia. Physiological races are also found in the populations of *F. oxysporum* f. sp. *vasinfectum.*

(c) *Symptomatology*

The initiation of symptoms of the disease appears on seedlings. Necrotic spots in the beginning appear on cotyledons; later on, leaves, reticulate necrosis observed along their veins. Affected leaves fall and plants dry out. The infection can occur in

Fig. 20.2 *Fusarium* wilt of cotton

stages of budding, flowering and later developing in vascular system of cotton. Affected tissue of conducting vessels of leaves, petioles, stalks and bolls becomes dark brown, a characteristic symptom of the disease. Wilting and drooping along with discolouration of vascular bundle while splitting the stem and root portion longitudinally is the typical symptom of *Fusarium* wilt. Sometimes infestation of nematode may facilitate the entry of this pathogen and can occur at any stage of the crop. Seedlings and small plants show yellowing and bronzing of cotyledons and leaves. The leaves lose turgidity, wilt, dry up and fall down. Once a field is infested, the fungus usually persists indefinitely (Wood and Ebbels 1972; Smith and Snyder 1975). Survival of the fungus in soils not planted with cotton for over 10 years has been documented (Smith et al. 2001) (Fig. 20.2).

(d) *Disease Management*

The disease can be reduced by deep ploughing during land preparation and exposure to solar radiation. Removal and destruction of infected plant debris and crop rotation with non-hosts are also useful in disease management. Seed treatment with Thirum 75%WS @ 3 g/Kg seed is recommended (CIBRC). Soil drenching with carbendazim @ 1 g/l of water around the plants helps in reducing soil inoculum (CIBRC).

20.2.1.3 Bacterial Leaf Blight of Cotton (BLB)

(a) *Distribution and Economic Importance*

The bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* (E. F. Smith) (Vauterin) is a disease of economic significance throughout the world. The pathogen can infect the cotton plants at all growth stages infecting leaves, bracts, bolls and stems and causes black arm, seedling blight, angular leaf spot and boll lesions (Verma 1986, 1995). Mishra and Krishna (2001) reported 1% and 27% yield losses depending on the cultivar and crop age. Under natural bacterial blight infection, boll yield losses up to 35% have been reported by Raj (1988). Very few sources of resistance to bacterial blight have been identified (Tyagi and Olngotie 1988) (Fig. 20.3).

Fig. 20.3 Bacterial leaf blight (BLB) in cotton

(b) *Symptomatology*

Bacterial blight is an important seed-borne bacterial disease of cotton and consisted of four phases, viz. seedling blight, angular leaf spot and vein blight phase, black arm phase and boll rot phase. The symptoms appear as dark green, water-soaked, angular lesions varying from 1 to 5 mm across on leaves, cotyledons and bracts. Spots are more obvious on the lower leaf surface with dark lesions which become black with age. Premature leaf shedding leads to extensive defoliation. Symptoms are usually more prominent on lower leaves (Verma 1986, 1995).

(c) *Aetiology*

Causal Organism: Bacterial blight of cotton is caused by a bacterium *Xanthomonas axonopodis* p.v. *malvacearum.* It is a gram-negative rod-shaped, non-spore-forming, aerobic, capsule-forming bacterium, which produces yellow colonies in culture medium. It occurs singly or in pairs and is motile by one polar flagellum (Yadav 2016). The pathogen can remain as slimy mass inside the seed or on the fuzz. The disease may be carried over through infected leaves, bolls and twigs on the soil surface. The secondary infection is through irrigated water and wind.

(d) *Management*

Cultivate recommended improved resistant hybrid varieties. Seed treatment is very important before sowing with sequence order as chemical fungicide, insecticide and bactericide/biofertilizers. According to Zohurul Islam et al. (2003) in the absence of resistant cultivars, antibiotics serve as the plant protection measures for controlling this disease.

For the management of seed-borne bacterial disease, seed treatment with Carboxin 75%WP @ 1.5 g/Kg of seed or Carboxin 37.5% + Thiram 37.5% @ 2.5 g/Kg of seed is recommended (CIBRC). For foliar application, spraying of copper oxychloride + streptocyclin (25 g + 1 g) mixed in 10 l of water is recommended (CIBRC). Again, spraying of same dose of Copper oxychloride + streptocyclin (25 g + 1 g) in 10 l of water at the interval of 15–20 days if BLB infection reappears in field (CIBRC).

20.2.2 Jute (*Corchorus capsularis* L and *C. olitorius* L)

Jute is the second most important fibre crop after cotton. It is grown mainly in the southeast Asian countries like India, Bangladesh, Nepal, China, Indonesia, Thailand, Myanmar and a few South American countries. Unlike cotton, the fibre is extracted from the stem and hence known as bast fibre. Jute is attacked by a number of pathogens. Among the seed-borne fungal diseases; stem rot, anthracnose and black band are of major importance and cause substantial yield loss in jute. Among these, *Macrophomina phaseolina* (Tassi) Goid. is the most devastating fungal pathogen

(Roy et al. 2011a, b). Baxter (1960) reported that *Macrophomina phaseolina* and *Diplodia corchori* were the most predominant seed-borne pathogens of jute. Black band and anthracnose may also cause significant damage to the crop (Mathur et al. 1992). The other diseases are seedling blight, leaf blight, anthracnose and leaf mosaic (Roy et al. 2011a, b; Meena et al. 2014). Among them stem rot of jute is most devastating.

20.2.2.1 Stem Rot of Jute

(a) *Distribution and Economic Importance*

Jute stem and root rot caused by fungus *Macrophomina phaseolina* is most economically important and devastating disease of both the cultivated species *C. capsularis* L. and *C. olitorius* L. (Meena et al. 2014). The pathogen is not only responsible for yield loss but also deteriorate the quality of fibre and seeds (Fazli and Ahmed 1959; Ahmed 1968; Roy and Mandal 1976; Biswas et al. 1980). The losses due to this devastating seed-borne disease of jute are 8–20% depending on the severity from year to year (Ahmed et al. 1985). Stem rot alone can cause 10% yield loss annually (Ahmed 1968). From seed germination till harvest, all the plant parts and growth stages are vulnerable to the disease and cause severe incidence up to 35–40%. Because of wide host range and climate changing scenario, the management of this pathogen has become more difficult (Ghosh 1983; Mandal 1990; Meena et al. 2014).

(b) *Symptomatology*

The disease may cause damage at any stage of growth to jute plants. Stem rot may completely kill the jute plants resulting in gaps in the field (Ahmed 1966). Due to its seed-, soil- and air-borne nature, both seed and fibre crops continuously get damaged starting from germination to maturity. Very young seedlings, about 2 inch height, die with the "damping-off" symptoms in which the hypocotyls become soft and rot completely. In diseased seedlings an infectious mycelium is found growing inside the tissues of the young stem. The primary symptoms appear either on the hypocotyls in the form of lesions near the collar or at the node as thin blackish-brown to black streaks (De 2013). Under wet conditions the younger seedlings damp-off. But when conditions are dry and the seedlings have reached a three-leaf stage, they develop blight resulting in the shedding of the leaves and the death of the plants. Sclerotia are, however, formed in all the parts of the seedlings.

(c) *Aetiology*

Macrophomina phaseolina (Tassi) Goid is the pycnidial stage of the pathogen. The pycnidial stage coupled with sclerotial stage are primarily responsible for the infection of disease in jute (Ghosh 1983; Mandal 1990). Seed is an important source of primary inoculum than infection through soil (De 2013). Stem rot epidemic can be measured from primary infection, as secondary infection is usually four times.

March sown crop is more prone to stem rot and root rot. Continuous cloudy atmosphere, heavy rainfall high atmospheric humidity, air temperature below 32 °C and soil temperature below 30 °C favour infection (De 2013).

(d) *Diseases Management*

Treatment with 50% N: P: K + seed treatment with *Azotobacter* and PSB @ 5 g/Kg + *T. viride* (seed treatment @ 5 g/Kg of seed and soil application @ 2 Kg/ha at 21DAS) + *P. fluorescens* spray @ 0.2% at 45DAS was found to be a more superior eco-friendly treatment in controlling stem rot and root rot disease of jute (Meena et al. 2014).

20.2.3 Ramie (*Boehmeria nivea* L. Gaud)

Ramie (*Boehmeria nivea* L. Gaud) is popular as an oldest and valuable fibre crop of the world and is well known for its world's strongest and longest natural fibre. It consists of good durability, highest strength and absorbency. Ramie fibre is used to prepare several products such as thread, packing materials, fishing nets, filter cloths, paper, etc. In India it is a traditional fibre crop of north-eastern states. As the crop has the semi perennial nature it stands in the field throughout the year. Hence due to seasonal variation the incidence and occurrence of diseases and insect pest are varying. Now-a-days due to change in climatic condition and also increase in the area under cultivation, this crop is observed to be suffering heavily from various diseases and insect pests which are causing heavy losses in yield and quality of this valuable fibre. Important diseases of ramie are as follows.

20.2.3.1 Anthracnose Leaf Spot of Ramie

(a) *Economic Importance*

Wang et al. (2011) reported that from June 2007 to September 2010, typical anthracnose symptoms were observed in cultivated ramie fields in China, with the diseased area estimated to be more than 10,000 ha. Severe infection results in premature defoliation. Ramie fibre yield was reduced by 20% on average in some fields but up to 55% losses were also recorded. Disease incidence was observed during November to March where the temperature ranges from 12 °C to 26 °C. The infection was highest during October to January (Gawande and Sharma 2016).

(b) *Symptoms*

On young as well as on mature older leaves, symptoms include water-soaked small greyish brown spots. These spots spread and coalesce to form dark brown to purple, irregular large spot, which cover the whole leaf lamina. As the disease progresses, the spots turn grey in the centre with a brown margin. Infected foliage looks pale yellowish green with leathery texture. Also infected leaves show chlorosis

Fig. 20.4 Anthracnose leaf spot of ramie

symptoms, which later during advanced stages turns necrotic. This necrotic region increases and detaches very easily, and shot holes are formed (Gawande and Sharma 2016) (Fig. 20.4).

(c) *Aetiology*

The disease is caused by *Colletotrichum gloeosporioides*. On potato dextrose agar, *C. gloeosporioides* initially developed white colonies with orange conidial mass, and the colonies turned to grey or brown after 5 days of incubation. Conidia are single celled, colourless, straight, oval, and obtuse at both ends. Conidiophores are dense and setae were few and dark brown with one to two septa (Wang et al. 2011).

(d) *Management*

Apply fungicides judiciously, i.e. spraying of Propiconazole or Difenconazole @ 0.1% or Mancozeb @ 0.25% 15 days after previous harvest for management of foliar diseases (Gawande et al. 2016).

20.2.3.2 *Sclerotium* Rot (*Sclerotium* sp.)

Sclerotium sp. primarily attacks ramie stem, although it may infect any part of a plant under favourable environmental conditions. The first symptoms of infection are normally unnoticeable, these are dark-brown lesions on the stem at or just beneath the soil level; the first symptoms are progressive yellowing and wilting of the leaves. Ramie seedlings are very susceptible and die quickly once they become infected. Mustard like sclerotial bodies germinate very rapidly as soon as the favourable condition occurs in the field. Frequent rain followed by warm temperature (20–30 °C) is the most important factor for the spread of this disease (Gawande et al. 2011) (Fig. 20.5).

Fig. 20.5 *Sclerotium* rot of ramie

(a) *Management*

Proper field sanitation and removal and proper disposal of infected plants help in reducing the disease. Use disease-free planting materials or use of resistant cultivars. For management of *Sclerotium* rot disease, drenching of copper oxychloride @ 0.25% is effective. Dip the plantlets or stem cuttings in 0.2% solution for 2 min. before plantation (Gawande et al. 2016).

20.2.4 Flax (*Linum usitatissimum* L.)

Flax, also called linen, is a fabric produced from fibre extracted from the stems of the flax plant and is an important textile after wool and cotton. Common flax was one of the first crops domesticated by man. There are several diseases appearing on flax, but few of them deteriorate the quality of linen fibre. Among them wilt, rust and powdery mildew are important diseases of flax associated with seed.

20.2.4.1 *Fusarium* Wilt

(a) *Economic Importance*

Flax wilt is caused by *Fusarium oxysporum* scht. f. sp. *lini.* (Bolley) Sinder and Hansen. Continuous cropping in the infected field makes the soil sick. Sattar and Hafiz (1952) reported up to 80% losses due to wilt pathogen in the flax field. In severe cases crop is completely destroyed due to wilt pathogen.

(b) *Symptoms*

Early infections during seedling stage may kill the plant very soon after emergence whereas delayed infections leads to yellowing and wilting of leaves, followed by browning and mortality of plants. Roots of infected plants become ashy grey. Infected plants occur more commonly in patches but may also be scattered throughout the field. The fungus present in the sick field in the form of spores and mycelia, can survive for many years in organic matter and debris of flax in the soil. Spore

dispersal through wind and irrigation water may spread the fungus from one field to another.

(c) *Aetiology*

Flax wilt or *Fusarium* wilt is caused by the seed-borne and soil-borne fungus *Fusarium oxysporum* f. sp. *lini.* The fungus attack plants through the root portion at any growth stage during the cropping season and start infection within the water-conducting tissue of the root. This interferes with nutrient uptake and the warm weather aggravates the disease symptoms (Rashid and Kenaschuk 1993).

(d) *Management*

The most important control measure is the use of available resistant/moderately resistant varieties. Seed treatment with recommended fungicides may protect the crop from early infection at the seedling stage and helps maintain good stands and seedling vigour.

20.2.4.2 *Alternaria* Leaf Blight

(a) *Economic Importance*

It is caused by *Alternaria lini* and is more serious in India particularly northern part of the country. Leaf infection causes damage from 27% to 60%. However, bud infection causes up to 90% losses. Chauhan and Shrivastava (1975) reported 28–60% losses in flax from Kanpur.

(b) *Symptoms*

In most of the fields, sporadic incidence of the disease may be due to fungus *Alternaria lini* causing seedling and stem blight. Symptoms include brown lesions on cotyledons and lower leaves also brick-red lesions on the stem base of some plants. Seedlings growth may be stunted due to infection.

(c) *Aetiology*

The disease is caused by *Alternaria lini.* This pathogen, *Alternaria lini*, is a seed-borne pathogen.

(d) *Management*

This disease can be controlled by seed treatment with vitavax powder @ 2 g/Kg of seed (Singh et al. 2014).

20.2.5 Sisal (*Agave sisalana*)

Sisal is the most important fibre crop in tropical and subtropical areas in China and other parts of the world. Sisal hard fibre is widely used for various purposes such as manufacture of twines, ropes, sacks and carpets. There are only two economically important diseases of sisal which causes yield losses.

20.2.5.1 Zebra Disease

(a) *Economic Importance*

Zebra disease results in significant loss at the seedling stage and considerable reduction in yield worldwide. It is a serious threat to the main cultivar Agave hybrid No.11648 (H.11648) in which the infections give very poor grade fibre and often renders the crop unusable.

(b) *Symptoms*

Zebra disease starts initially with the leaf rot which later spread into the stem leading to the death of plant. Leaves with infested soil or water are the most important modes of infection. Wet spells or moisture in the soil are needed for fungal infection and during prolonged rainfall the disease can spread rapidly. Soon after contact with water the sporangia typically germinate and zoospores are released into soil (Gao et al. 2012).

(c) *Aetiology*

It is caused by *Phytophthora nicotianae* and mainly occurs in poorly drained soils.

20.2.5.2 Bole Rot

(a) *Symptoms*

Due to the infection of this fungus, the wet rot, which becomes yellowish-brown and soft with a pinkish margin, may cause collapse and death of plant (Gao et al. 2012). The fungus enters the base of the bole through an injury and causes a basal dry rot.

(b) *Aetiology*

Bole rot caused by the fungus *Aspergillus niger* is the most serious disease of sisal which enters through the injured leaf bases.

(c) *Disease Management*

The incidence can be managed by destruction of infected plant parts and harvest during dry weather conditions. Mancozeb 0.2% or Metalaxyl 8% + Mancozeb 64% formulation 0.2% and their combination in alternations are effective in reducing zebra diseases (Roy et al. 2011a, b).

20.2.6 Sunn hemp (*Crotalaria juncea*)

Sunn hemp is mainly grown for fibre or green manure, and leaves consisting of high protein are fed as a supplement to other poorer feeds. Due to its dense canopy, it is also used as a cover crop to reduce weed populations. Sunn hemp is infected by many diseases caused by viruses, fungi and nematodes, but they normally cause very less economic damage. The economically important diseases of Sunn hemp cultivated in India are anthracnose, caused due to *Colletotrichum curvatum* (Mitra 1934; Whiteside 1955), and a wilt, caused by *Fusarium udum* f. sp. *crotalariae* (Mitra 1934).

Seed treatments with fungicide and crop rotations are the most suggested and practiced disease control measures (Mitra 1934; Whiteside 1955). Sources of resistance to anthracnose have been reported by Dey et al. (1990).

20.3 Major Seed-Borne Diseases in Important Forage Crops

Forage crops like other field crops are susceptible to several fungal, bacterial and viral diseases. Forage crops may suffer heavy losses and causing heavy destruction in particular areas by certain diseases. Most of the forages that require climatic conditions favourable for crop growth also favour disease development. Hence, it is important to have detailed study of the diseases for suitable and effective management. This section explains the major seed-borne diseases of forage crops, symptomatology, economic importance, aetiology and management.

20.3.1 Berseem/Egyptian Clover (*Trifolium alexandrinum*)

20.3.1.1 Root Rot Complex

(a) *Economic Importance*

Wakelin et al. (2016) reported that losses due to root rot disease in clover in Southland and Canterbury of New Zealand costs $750 and $715 per ha per year, respectively, whereas the Waikato region estimated with $1506 per ha per year. The losses may vary according to location, environmental factors and cultivars in different cropping systems.

(b) *Symptomatology*

Initially, there is drooping of leaves and morbidity of few tillers of diseased plants under favourable weather conditions. The disease may appear in patches in the affected field. The severe crop damage occurs in the field with high relative humidity coupled with warm weather just after winter season.

(c) *Aetiology*

This disease is caused by *Rhizoctonia solani*, *Fusarium semitectum* and *Sclerotium bataticola.*

The primary infection is caused through mycelia, conidia, sclerotial bodies in the diseased plants left over in the field as well as infected seeds. The secondary infection is caused by irrigation water, wind-borne spores and mycelium from infected plants to healthy plants. The disease is more persistent at luxuriant vegetative growth stage of crop and longer cloudy days. The mycelial growth can be seen at collar regions and root zones.

20.3.1.2 Crown and Stem Rot

This is known as one of the most destructive disease of the berseem. The disease is caused by virulent fungal pathogen, *Sclerotium trifoliorum.* The disease is commonly known as crown and stem rot, but may also be known with several names like clover rot, clover sickness, etc. It has wide host range and vast distribution in several cropping systems. The occurrence of this disease has worldwide distribution in berseem or Egyptian clover cropping areas.

(a) *Symptomatology*

Initially the symptoms appeared on leaves as minute, light yellow to brown spots on leaves and petioles. As the disease progresses, the infection spreads rapidly on leaves and becomes dark grey to brownish in colour. Under favourable conditions, the whitish mycelium appears infecting crowns and roots of crop. Thereafter, the crown and lower parts of younger stems starts rotting, becomes soft and grey to brown in colour and extends up to root portion. Some part or heavily diseased portion of plants rots, wilts and eventually dry and dies. In case of high humidity; the infected leaves, stems and petioles get covered with white mycelium on diseased plants. This disease generally occurs in isolated patches in field. However, in case of high humidity and favourable low temperature, this causes heavy losses in the field. The stable or increased temperature ceases the disease progress and plants may recover.

(b) *Aetiology*

Causal organism: *Sclerotium trifoliorum*

Under high temperature, mass of mycelia gets converted into hard bodies i.e. sclerotia. Sclerotial bodies remain attached to diseased plant parts, stems and roots and soil. These sclerotial bodies act as source of infection. The infection is also caused by ascospores (perfect stage of the fungus). The secondary infection is caused by newly diseased plants to another plant by mycelium. High relative humidity, dense planting and warm weather favour the disease development. The sclerotial bodies mixed with seed lot may also cause the spread of the disease to newer areas.

20.3.1.3 Berseem Mosaic

(a) *Symptomatology*

Typical light to greenish mottling appears on the infected leaves along the veins and veinlets with distinct vein clearing. It may be chlorotic streaks or in patches. The infected leaves may turn yellowish-green in colour. The systemic infection may also be present in hosts and symptoms may appear after 3 weeks.

(b) *Aetiology*

The insect vector, *Aphis gossypii*, transmits the virus from diseased berseem to healthy berseem (60–70% seed transmission). It is also a sap transmissible virus. The cool and humid weather favours the disease spread.

20.3.2 Lucerne/Alfalfa (*Medicago sativa*)

(a) *Economic Importance*

Nutter et al. (2002) stated that in the United States, the foliar diseases in alfalfa cause significant negative damages on alfalfa yields. Several researchers reported that fungi, bacteria, viruses, phytoplasmas and nematodes caused potential damages to alfalfa crop production (Hampton et al. 1978; Teng 1985; Hanson et al. 1988; Leath et al. 1988; Campbell and Duthie 1990; Stuteville and Erwin 1990). The fungal diseases cause severe crop losses and significant quality reductions in forages (Gray 1983; Broscious et al. 1987; Broscious and Kirby 1988; Campbell and Duthie 1990; Stuteville and Erwin 1990).

20.3.2.1 Bacterial Wilt

(a) *Symptomatology*

There is stunting and yellowing of infected plant. The stunted plants are pale green to yellow. The florets become rounded at top with upward curling. During warm and dry weather, plants may wilt and die. First sign is drooping of leaves followed by wilting and death in case of severe infection. In winter season, the infection is severe. In diseased plant, there is discolouration of vascular tissues of tap roots with yellow to dark brown colour. The wilting results due to blocking of

vascular bundle by bacterial colonization and production of bacterial toxins and exopolysaccharides.

(b) *Aetiology*

This disease is caused by *Corynebacterium insidiosum* [Syn. = *Clavibacter* (*Corynebacterium*) *michiganense* subsp. *insidiosum*)]. The pathogenic bacteria survive in crop residues in soil and in field. The bacterial transmission in the field is carried out by irrigation water, flood water, agricultural implements and farm animals. The infected seed is also the major cause of infection. The infection is mainly caused during winter and humid condition. The bacteria enter through natural opening, cuts and injuries in roots and crowns. As the disease progress, there is rapid multiplication of pathogenic bacteria in crowns and stems.

20.3.2.2 Downy Mildew

(a) *Symptomatology*

The upper leaf surfaces are covered with whitish mycelium which appear as mottled light to yellow coloured. Greyish to blue-coloured mycelia and spores can be seen on underside of infected leaves. Higher sporulation occurs in morning hours with high relative humidity. The tender buds and leaves become infected by fungus and there is occurrence of malformed yellow leaves. In severe infection there is drooping of leaves with reduction in quality and thereby yield.

(b) *Aetiology*

This disease is caused by *Peronospora trifoliorum.* Infection of downy mildew is noticed in winter season of crop. The pathogen requires low temperature and high humidity for production of spores and infection. The young seedlings are highly susceptible under favourable weather.

(c) *Disease Management*

The best option for control of this disease is development and use of resistant varieties. Downy mildew-affected crops should be cut on the early side of bloom to save as many of the leaves as possible. Since the fungus may be seed-borne, it is advisable to obtain seed from a healthy, disease-free crop.

20.3.2.3 Black Stem

(a) *Symptomatology*

Whole plant parts may be affected by the disease. The disease spots are small brown to black coloured, often with pale yellow halo. The spots on stems and petioles become dark brown with yellowish margin. The brownish to black-coloured

microsclerotial bodies can be seen on petioles and dead parts. The infected parts become withered and weak.

(b) *Aetiology*

This disease is caused by *Ascochyta imperfecta*. The primary infection is caused by crop residues and infected seeds with mycelium and pycnidia. The low to moderate temperature and high relative humidity are ideal for leaf spot and black stem development. The infection to flowers and beans occurred during precipitation. The secondary infection is caused by infected plant parts, rain water and seeds.

20.3.2.4 Spring Black Stem

(a) *Symptomatology*

All the foliar parts of plant may get infected. The initial symptoms are small, light brown to dark spots on leaves, petioles and stems. As the disease progresses, the disease spots on stems enlarge, get coalesced and appears black in colour. The entire petioles, stems may get covered with black mycelia. The severely infected plants wither and dry. The infected foliage turns yellow and dry off.

(b) *Aetiology*

Causal organism: *Phoma medicaginis* (*Ascochyta imperfecta*). The primary infection is caused through diseased crop debris in the field, seed coats and soil. The cooler and high humidity conditions are ideal for the disease progress. The secondary infection spreads through infected crop debris, rain water, winds and insects. The pathogen also survives on clovers, common vetch etc.

20.3.2.5 Alfalfa Mosaic

(a) *Symptomatology*

There is general mottling and typical yellow streaks between leaf veins. The leaves are mostly stunted in growth, crinkled and in case of severe infection the whole plant gets stunted. In winter season, the severity of infection is more and reduces as temperature increases slowly towards summer season.

(b) *Aetiology*

This disease is caused by *Alfalfa mosaic virus*. This disease is more severe in cooler regions of cultivated areas. The symptoms vary considerably according to viral strains. The virus transmission occurs mainly by aphids, by seeds or by pollen to the seed (Bisby et al. 2008) and it can infect other alternate hosts and leguminous forage crops.

20.3.2.6 Anthracnose Disease

(a) *Symptomatology*

The infected plants appear as yellowish to grey coloured, scattered in the field which are visible from the distance. The infected tender stems may bend due to the disease attack. The lower part of the stems appears grey to brownish in colour. Under favourable weather, the centre of the disease spots may appear as black conidial fruiting structure of anthracnose pathogen. The disease spots further coalesce to form large necrotic spots. The fungus may infect the crown portion of the plant and cause the crown rots.

(b) *Aetiology*

Causal organism: *Colletotrichum trifolli.* The pathogen overwinters in infected crop residue and infected seeds and may be seed-borne. The warm and high relative humidity intensifies the disease condition. Though the disease appears at any stage of crop, but is most prevalent in the seeding stage. The secondary infection is caused by conidia in the diseased plants and by rain water splashes.

20.3.2.7 Rust

(a) *Symptomatology*

Initially, reddish to brown eruption of the spore masses develops through the epidermal layer of leaf tissues and brown powdery pustules appear on leaf surface. Rarely, yellow halo may be seen around the diseased spots. These pustules are commonly seen over the leaves surface. The pustules extend over the stems and petioles, thereby reducing the photosynthesis and quality of the forage.

(b) *Aetiology*

This disease is caused by *Uromyces striatus.* The rust fungus overwinters in the cool cropping season. The high relative humidity and temperature range between 21 °C and 29 °C is most favourable for the disease development. If the harvesting is delayed the stems and petioles may get severely infected.

20.3.3 Sweet Clover (*Melilotus indicus*)

(a) *Economic Importance*

The foliar fungal diseases are most common but do not cause severe damage to herbs. However, in case of severe infections, these diseases significantly reduce yield as well as crude protein quality and content in sweet clover.

20.3.3.1 *Rhizoctonia* Blight

(a) *Symptomatology*

Initially, greyish to brown small patches may appear on the stems which coalesce or enlarge to form large patch. There may be severe blight and defoliation of the crop in severe infection under favourable weather. The black discolouration is seen on the stems due to the production of pycnidia or spores during the crop season.

(b) *Aetiology*

This disease is caused by *Rhizoctonia solani.* The infected seeds or diseased crop debris in the field is the primary cause of the infection. The fungal pathogen overwinters in the field. The black-stem disease may attack plants less than one year old but more often the greatest amount of damage occurs during the second year. On some varieties the infection may spread to the small stems, young leaves, flowers or seed. When this condition develops the damage may be considerable. The secondary infection is caused by diseased or infected plant or by fungal spores in the field.

20.3.3.2 Root Rots and Seedling Blights

(a) *Symptomatology*

This is the most destructive disease of sweet clover caused by *Sclerotium rolfsii* and *Fusarium solani.* The young seedlings are severely affected and cause rapid wilting and blights. There is wilting and severe rotting of the crowns. The tap roots are disintegrated and rotted and the infected plants may be easily pulled out of soil. In initial infection, the upper tips of the plants show slight wilting.

(b) *Aetiology*

Causal organism: *Sclerotium rolfsii* and *Fusarium solani*. The primary infection is caused by diseased crop debris left in the field. The secondary infection takes place by sclerotia, conidia and/or mycelia of diseased plant to healthy plants in field.

20.3.4 Shaftal/Persian Clover (*Trifolium resupinatum*)

In warm and humid weather, foliar diseases especially rust (*Uromyces trifoliirepentis*) and clover rot (*Sclerotinia trifoliorum)* causes severe problems.

20.3.4.1 Root and Crown Rot

(a) *Symptomatology*

In *Rhizoctonia* infection, the brown to rusty, dry sunken lesions or spots generally appear on the stems and roots near soil zone. Additionally, the lateral roots may be discoloured and decayed. The seedlings are severely infected and become

yellow, stunted and therefore wilted. The collar region infection is most common which cause stunting and killing of plants. In *Sclerotium* infection, the seedlings are withered and dry. There is discolouration and rotting of collar region by the fungus, thereby leading to death of plants. In *Fusarium* infection, there is general yellowing, withering, stunting and wilting of the infected plants. The vascular tissues get discoloured and blocked by pathogen colonization.

(b) *Aetiology*

This disease is caused by group of fungal pathogens, *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani*. The primary infection is caused by infected seeds or by the diseased or infected plants in the field. The disease may appear in patches in the field. The environmental and edaphic factors like high soil moisture, high organic matter in soil, warmer soil temperatures favour the disease development. The nematode injury favours the disease condition.

20.3.4.2 Rust Disease

(a) *Symptomatology*

The symptoms appear on leaves, tender foliage and stems. Commonly, light to dark brown-coloured spots or pustules can be observed on upper surface of leaves. The rusty urediniospores are brownish, circular and scattered over leaf surfaces. Whereas, the teliospores are dark brown in colour and little raised. There is withering and drying in case of severe infection.

(b) *Aetiology*

This disease is caused by *Uromyces trifolii-repentis.* The rust fungal pathogen has complex life cycle with five spore stages and require two different host plants to complete life cycle. The fungus is carried by seeds, infected crop debris and alternate hosts. The primary infection is initiated by wind spread spores. The secondary infection takes place through urediniospores to crop plants and other hosts. High relative humidity favours the rapid development of the disease.

20.3.5 Methi/Fenugreek (*Trigonella foenum-graecum*)

(a) *Economic Importance*

The powdery mildew caused by *Erysiphe polygoni* and *Cercospora* leaf spot caused by *Cercospora traversiana* are the major fungal diseases (AAFRD 1998), which may cause heavy losses under favourable weather conditions. The powdery mildew caused by *E. polygoni* seriously damage crop production (Prakash and Saharan 2000; Jongebloed 2004). The leaf blight caused by *C. traversiana* and wilts caused by *Fusarium oxysporum* and *Rhizoctonia solani* result in serious reduction

of crop yields in Australia (Jongebloed 2004). In Haryana, state of India, sowing of seeds in mid-October as compared to November end shows 30% reduction in yield damage by *E. polygoni* and *Leveillula taurica* (Sharma 1999).

20.3.5.1 *Rhizoctonia* Blight/Charcoal Rot

(a) *Symptomatology*

The discolouration and cankers on stems appear on the young seedlings. The leaves may wither and wilt, thereby drooping of the leaves occur. Under favourable weather conditions, numerous black sclerotia develop in diseased tissues.

(b) *Aetiology*

The disease is caused by *Rhizoctonia solani*. The primary infection is caused by diseased or sclerotial fruiting bodies in soil or infected plants in the field. The pathogen has wide host range infecting many field crops. The secondary infection is caused by conidia/sclerotial bodies in the diseased crop debris in the field or infected plants.

20.3.5.2 Root Rots and Wilts

(a) *Symptomatology*

The affected plants of one month show yellowing of leaves. The roots are poorly developed whereas root hairs may rot or may not be formed. The disease incidence is severe under high soil moisture in water logged conditions. The rotted plants may be easily pulled out from the soil. The vascular discolouration and disintegration of vascular tissues can be seen in infected plants by *Fusarium solani*.

(b) *Aetiology*

Causal organism: *Rhizoctonia solani, Fusarium solani, R. bataticola.* The primary infection is caused by diseased plant, sclerotial bodies or infected crop debris. The secondary infection is caused by newly infected plants, mycelia in diseased crop debris and sclerotia in soil.

20.3.5.3 *Cercospora* Leaf Spot

(a) *Symptomatology*

The round, water-soaked lesions with chlorotic halo appear on the leaves due to fungal infection. The disease spots extend to form necrotic patches on the leaves. The spots on the pods seen as discoloured brownish areas.

(b) *Aetiology*

This disease is caused by *Cercospora traversiana*. The primary infection caused by infected seeds, leaves and debris in the field. The secondary infection takes place through conidia from infected plants to healthy plants.

20.3.6 Lobia/Cowpea (*Vigna unguiculata*)

(a) *Economic Importance*

Adegbite and Amusa (2008) stated that the pathogens *Colletotrichum* sp., *Cercospora canescens, Pseudocercospora cruenta, Protomycopsis phaseoli, Rhizoctonia solani, Choanephora curcubitarum, Sclerotium* and charcoal rot have congenial environment in south-western Nigeria for survival and disease development in cowpea causing significant crop yield losses. In Georgia, Toler et al. (1963) assessed the economic importance of several cowpea diseases and concluded that southern blight was responsible for an economic loss of <1%.

20.3.6.1 *Sclerotium* Blight

(a) *Symptomatology*

Initially there is yellowing and withering of leaves and thereby sudden wilting of the whole plants. The affected plants show browning of stems and branches, and water-soaked lesions develop at collar region of host plants. In warm and humid weather, the stems may be covered with white mycelial growth with small light to dark brown sclerotial bodies.

(b) *Aetiology*

This disease is caused by *Sclerotium rolfsii.* The primary infection is caused by infected seeds, mycelia and/or sclerotia present in the crop debris in field soil. The secondary infection spreads by infected plants and sclerotial bodies in field to healthy plants.

20.3.6.2 Wilt

(a) *Symptomatology*

The affected plants have stunted growth; yellow leaves are withered and dry. The foliar portion of diseased plants becomes necrotic near basal portion of infected hosts. The brown to reddish, sometimes black streaks may be seen on roots which coalesce with disease progression and can be seen above ground level at stems. The vascular tissues may be discoloured and disintegrate due to fungal colonization and blocking of tissues.

(b) *Aetiology*

This disease is caused by *Fusarium oxysporum.* The primary infection is caused by infected seeds and diseased crop debris present in soil. The warm, low fertility and poor drained soil make the plants more susceptible to attack by the pathogen. The secondary infection is caused by spores carried by irrigation water or rain splashes from infected plots in the field.

20.3.6.3 Cowpea Mosaic

(a) *Symptomatology*

The typical mosaic pattern of disease appears on the leaves of infected plants by *Cowpea mosaic virus*. The younger leaves are highly susceptible to mosaic. The symptoms may be irregular with light to dark yellow–green pattern on the leaves. The different viral strains may cause variable symptoms like stunted, malformed, thickened and distorted morphology of the leaves. The diseased plants look stunted in growth, affecting the normal growth of plants and may fail to produce the pods. Similarly, if the infection occurred on younger plants, it does not produce pods. The most common mosaic virus disease on cowpea is of potyvirus group transmitted by aphids.

(b) *Aetiology*

The CMV is transmitted by aphids as well as sap transmission. The virion is also seed-borne. The alternate hosts from leguminous family also act as source of infection to several strains of CMV.

20.3.7 Guar/Clusterbean (*Cyamopsis tetragonoloba*)

(a) *Economic Importance*

The fungal pathogen, *Fusarium solani*, is a devastating pathogen of cluster bean seeds causing wilt disease which reduce quality and yield resulting in heavy economic losses (Pareek and Varma 2015). Guar (*Cyamopsis tetragonoloba*) suffers from several diseases which reduces the quality and yield performance resulting in heavy economic losses (Chand and Gandhi 1978; Pareek and Varma 2014).

20.3.7.1 Blight and Root Rot

(a) *Symptomatology*

The pathogens present in the seeds may cause rotting of the emerging seedlings in the soil and death of the young seedlings by blights and root rots. The emerged seedlings, hypocotyls and stem may appear with brown to reddish water-soaked lesions, brown in colour, and there may be rapid death of seedlings. In *Rhizoctonia*

and *Sclerotium* infection, the whitish mycelium may appear on collar and stems in warm and humid weather. The *Fusarium* infected seeds may rot and young seedlings appear as stunted, yellow and dry.

(b) *Aetiology*

This disease is caused by *Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium* spp. The primary infection is caused by infected seeds, conidia, sclerotia and/or diseased plant debris in the soil. The secondary infection is caused by spores/conidia, sclerotia carried by rain or irrigation water and infected plants in the field.

20.3.8 Rice Bean (*Vigna umbellata*)

(a) *Economic Importance*

The rice bean is a legume grown especially for food grain. The diseases do not cause severe losses in this crop. However, the diseases like *Rhizoctonia* blight, *Cercospora* leaf spot, powdery mildew, rust and bacterial blight may cause huge losses under favourable environmental conditions in major cropping areas.

20.3.8.1 *Rhizoctonia* Blight

(a) *Symptomatology*

Two types of disease symptoms occur at seedling (collar rot) and vegetative stage (web blight). Because of wide host range of the pathogen, development of disease resistant cultivars are difficult. The water-soaked, brown to black lesions develop at collar region with round-shaped sclerotia. The affected plant leaves turn yellow, defoliate, wilt resulting in death of whole plant. In web blight symptoms, small, round, grey to brown spots may develop and coalesce. The irregular diseased lesions are characterized by water-soaked areas. The whitish mycelial growth can be seen on stems and infected leaves in warm and humid weather.

(b) *Aetiology*

This disease is caused by *Rhizoctonia solani.* The primary infection is caused by infected seeds, sclerotia and diseased crop debris in the field. However, the secondary infection is caused by infected plants to healthy plants in the field.

20.3.8.2 Root Rots

(a) *Symptomatology*

The emerging seeds may rot by fungal pathogens in the soil in warm and humid weather. However, post emergence, stems may appear with brown to black water-soaked lesions and girdling at collar or basal part near soil line in *Sclerotium*

infection. The yellowing, browning of foliage and rotting finally result in death of whole plant. The white mycelial growth along with light brown to black-coloured sclerotia may be seen on diseased plants.

(b) *Aetiology*

Causal organism: *Rhizoctonia solani*, *Sclerotium rolfsii.* The initial infection is caused by seed-borne mycelia, sclerotia/mycelia present in diseased plants or in the field soil. The secondary infection is caused by infected plants, mycelia and sclerotia to healthy plants in the field.

20.3.8.3 Powdery Mildew

(a) *Symptomatology*

The fungal pathogen infects the leaves, tender stems and developing pods. It appears as powdery mycelial mass covered on the surface of infected plant parts. In case of high severity, there is shrivelling and distortion of affected plant parts. The diseased leaves curl, turn yellow to brown, dry and often defoliate. In heavy infection, pods may not be formed, if formed they are of small size and malformed.

(b) *Aetiology*

This disease is caused by *Oidiopsis taurica.* The primary infection takes place through infected and diseased plant parts in field. The disease may also be seed-borne. The secondary infection is caused by spores spread by winds and water. The temperature range of 20–35 °C with high relative humidity favours the disease.

20.3.9 Oat (*Avena sativa*)

(a) *Economic Importance*

Oats are attacked by many pathogens causing several diseases. The economic losses may be heavy as it reduces the quality of grain, forages and total yield. The ideal environmental conditions favour rust development at milking and before grain filling stage, likely to cause heavy losses.

20.3.9.1 Bacterial Blight

(a) *Symptomatology*

Initially sunken, water-soaked yellow to brown spots are seen on the foliar parts of the plants. These spots later enlarge into large blotches or as strips which in turn convert to brown rusty to black in colour.

(b) *Aetiology*

This disease is caused by *Pseudomonas striafaciens.* The pathogen remains in diseased crop residues in field and in seeds. Under favourable conditions, it causes infection to the new seedlings. The secondary infection spreads by winds, irrigation waters and splashing rains. The bacterial pathogen enters through stomatal openings, insect damage and injury caused by winds and intercultural operations.

20.3.9.2 Black Loose Smut

(a) *Symptomatology*

The young seedlings are infected during the germination of seeds and seedling stage. The infection is systemic and latent till the development of heads. The grains are colonized by fungal mycelium and filled with black powdery spores. As the disease progresses, almost all spikelets are infected and turns black.

(b) *Aetiology*

This disease is caused by *Ustilago avenae*. The fungal spores are carried on the surface of the seeds and underside of seed hulls. The primary infection is caused by infected seeds. However, the secondary infection is caused by sporidia carried by water and winds which remains dormant in the soil.

20.3.9.3 Covered Smut

(a) *Symptomatology*

The seed-borne infection is the principal cause of disease which occurs with germination of seeds into the young seedlings. The infection is systemic and latent. The symptoms appear during heading stage. The pathogen is less destructive to hulls as compared to loose smut pathogen. The spore mass is darker and hard as compared to loose smut.

(b) *Aetiology*

This disease is caused by *Ustilago kolleri.* The seed-borne spores are the primary cause of infection which remains adhering to the surface of seeds. The spore balls break during infection under ideal condition. The secondary infection is caused by sporidia dormant in the soil.

20.3.9.4 Root Rots

(a) *Symptomatology*

The infected plants are stunted with brown- to purple-coloured leaves. The infection in the field occurs in patches or as scattered. The infected leaves near the soil

zone appears as light to dark brown in colour. The internal tissues of the crowns are discoloured. The root rots are more severe under drought and insect pest damage.

(b) *Aetiology*

The root rots are caused by group of pathogens, viz. *Fusarium* spp., *Rhizoctonia solani*, *Helminthosporium* spp. and *Bipolaris* spp. Diseased crop residues, conidia and sclerotia in the field are the major cause of primary infection. The secondary infection is caused by infected plants to healthy plants in the field.

20.3.10 Barley (*Hordeum vulgare*)

(a) *Economic Importance*

Fusarium head blight causes heavy damage by reducing quality and yield. The seed size becomes small, shrivelled and white to pale coloured. The protein content lowers in seeds or grains and ultimately affects the yields. The mycotoxins content in the grains and contaminated seeds fetch lower price and results in heavy losses (Matny 2015). The yield losses ranging from 35% to 40% are commonly reported. In scalds, 1–10% losses are commonly observed.

20.3.10.1 Black Smut

(a) *Symptomatology*

The infection occurs during the seedling development but the visible symptoms appear when the heads are developed. The infection is latent and systemic. The kernel is ramified with mycelium mass and replaced with black mass spores. The symptoms range from loose smut to covered smut.

(b) *Aetiology*

This disease is caused by *Ustilago nigra.* The primary infection is caused by diseased plant debris, infected seeds and through soil in the field. The secondary infection caused by wind carried spores to healthy seeds through water and threshing.

20.3.10.2 Covered Smut

(a) *Symptomatology*

The infection by the fungal pathogen is caused during the seed germination and seedling stage without showing any visible symptoms. However, the symptoms appear during head development. The kernels are filled with grey to black mass of the spores, thus replacing the inner content of the kernels.

(b) *Aetiology*

This disease is caused by *Ustilago hordei*. The spores remain dormant in the infected heads and kernels. The head membrane breaks down during harvesting and disseminates to healthy seeds in the field. The spores remain viable in injury or cracks of seeds and hulls formed during threshing. The secondary infection is caused by sporidia present in the diseased plants in field soil.

20.3.10.3 Ergot

(a) *Symptomatology*

The fungal pathogen affects the cereals crops, grasses and forage crops. The disease symptoms are noticed as dark brown to black elongated, hard sclerotial bodies in the infected grains, heads or spikelet of hosts. The infection occurs in patches or scattered and does not cause severe loss in yield. Generally, the quality of the produce and grains are lowered. The alkaloids present in the sclerotia may cause poisoning to livestock animals. The seeds are replaced with ergot bodies and some kernels may be found as empty.

(b) *Aetiology*

This disease is caused by *Claviceps purpurea*. The primary infection is caused by sclerotial bodies present in the crop debris in soil. As the disease progresses, the secondary infection is caused by the spores carried by winds and infect the crop flowers. The young heads and maturing seeds produce "honey dew" symptoms, which attracts insects and the spores are also carried by them. At the time of seed setting the seeds are replaced with hard mass of mycelia or sclerotial bodies at maturity. These ergot bodies survive in the soil for long periods and are carried from one season to another. Among the cereals, oats are less infected.

20.3.10.4 Common Root Rot

(a) *Symptomatology*

In the humid weather, the seeds in the developing heads are infected by *Helminthosporium* which may develop shrivelled, malformed and black kernels. The black point infections are mostly noticed near the embryo or at the end of seed, hence the name given as black point. Thus, the infected seeds when sown may cause seedling blights. The young seedlings appear as yellow, stunted, with discoloured black roots and ultimately result in death of plants.

(b) *Aetiology*

This disease is caused by *Cochliobolus sativus* (Syn.= *Helminthosporium sativum*). The primary infection results from the mycelia or spores present in the

infected seeds and crop debris in soil. The secondary infection is caused by irrigation water, winds and splashing rain water.

20.3.11 Jowar/Sorghum (*Sorghum bicolor*)

(a) *Economic Importance*

In India, Das and Patil (2013) estimated an average loss of 3000–5000 million rupees every year by sorghum grain mold. In favourable condition, the yield loss by rust range from 29% to 65% (Bandyopadhay 1986; Hepperly 1990). The downy mildew systemic infection ranged from 2% to 20% as per severity, cultivars and environmental conditions. In the United States, the estimated US$ 2.5 million losses were found to be caused by downy mildew (Frederiksen et al. 1969).

20.3.11.1 Grain Moulds

(a) *Symptomatology*

The symptom varies according to pathogen involved in moulds and inoculums. Severity of the grain moulds is more at crop stage such as flowering and grain development under warm, high humid weather and rains with wind. There are many fungal pathogens involved in the grain moulds. The most common genera are *Aspergillus*, *Fusarium*, *Phoma*, *Curvularia* and *Alternaria*. The *Fusarium* spp. develops whitish to pinkish-coloured mycelia in diseased parts. Whereas, *Curvularia, Phoma* and *Alternaria* turn to grain black.

(b) *Aetiology*

The primary infection is caused by spores or conidia present in the crop debris in the field. They remain dormant as pathogen as well as saprophytes. The secondary infection spreads by wind, water, birds and insects in crop field. The rainy weather at flowering and grain filling stage favours the grain mould development.

20.3.11.2 Charcoal Rot

(a) *Symptomatology*

It is major disease commonly observed in dry land sorghum cultivated areas. The disease is most prevalent on hybrid sorghum varieties. When the crop is under moisture stress before flowering followed by hot and dry weather, the disease occurs. The diseased stalks may show internal discolouration and breaking of tissues. The splitting of the stalks may show tough vascular tissues. Often, blackening of the affected tissues is seen.

(b) *Aetiology*

This disease is caused by *Macrophomina phaseolina.* The primary infection is caused by conidia and microsclerotia present in the infected crop debris left over in the field, which may survive for several years in dry land soil. The soil temperature of 27–35 °C for 15–20 days favours the disease. The secondary infection is caused by conidia and microsclerotia of infected plants to healthy plants in the field.

20.3.11.3 Downy Mildew

(a) *Symptomatology*

The pathogen causes the infection through young shoots and tender tissues as systemic infection by oospores and/or conidia. When unfolding of leaves takes place, they appear as greenish to yellowish in colour. The lower side of the leaves show whitish mycelial growth composed of sporangiophores and sporangia. The chlorotic patches with downy mycelial growth are observed on young three to four leaves, whereas the strips or streaks are seen on subsequent leaves as disease progresses. Later, they develop necrotic patches releasing oospores with shredded leaves.

(b) *Aetiology*

The causal agent of the disease is *Peronosclerospora sorghi.* The primary infection is caused mostly through oospores, which are carried on the seed surface or which survive in the field soil, leading to systemic infection. Whereas, secondary infections are caused by wind-borne sporangia spread from infected plants. The high relative humidity, light precipitation and cool nights (21–23 °C) favour the disease development.

20.3.11.4 Anthracnose Disease

(a) *Symptomatology*

The two types of symptoms are produced by fungal pathogen as leaf spot or lesion, commonly known as anthracnose and another as stalk rot or red rot. The disease spot is characterized by white centre with reddish to brown margin. The minute black-coloured acervuli are often observed on the centre of the disease lesions. Typically, round cankers may be seen in florets, which is the external characteristic of red rot. Internal tissues of the infected stem show discolouration and disintegration with streaked pattern.

(b) *Aetiology*

This disease is caused by *Colletotrichum sublineolum.* The primary infection is by means of infected seeds and diseased plant debris in soil. The secondary

infection is caused by air-borne conidia and rain splashes. The temperature range of 28–30 °C with high relative humidity is congenial for disease progress.

20.3.11.5 Loose Smut

(a) *Symptomatology*

Before, the appearance of ear heads, the infected plants look stunted with thin stalks and less tillers. The ear development stage comes earlier than normal plants. The glumes in florets are overgrown than healthy plants. The infected sori are covered by thin membrane which may rupture early to release the spores.

(b) *Aetiology*

This disease is caused by *Sphacelotheca cruenta.* The primary infection is caused by diseased crop debris left over in the field and infected seeds (externally). The secondary infection is caused by winds and rain splashes. The acidic soil condition with temperature range of 20–25 °C favours the disease development.

20.3.11.6 Ergot

(a) *Symptomatology*

The first visible symptom on the plants appear as honey dew like secretion from the infected inflorescence. The infection is in single spikelets of florets. Under favourable environmental condition, the infected spikelets develop long, slender to curved, light brown-coloured sclerotia. Often, the honey dew secretion is colonized by fungus, *Crerebella sorghivulgaris*, which gives a black-coloured appearance of ear heads.

(b) *Aetiology*

The causal agent of the disease is *Claviceps africana.* The primary infection is caused by dormant sclerotia which germinate in the field releasing ascospores to cause infection to ovary in the inflorescence. The secondary infection takes place through conidia carried by air, rain splashes and insects. High precipitation, cloudy weather, cool nights and high relative humidity during flowering is highly congenial for disease development.

20.3.11.7 Root and Stalk Rots

(a) *Symptomatology*

The *Fusarium* stalk rot fungus cause rotting of roots, basal soil zone internodes and crown. There is stunting and wilting of plants. When the infected stalk is open, the internal tissues appear as light pink to reddish with disintegration of pith.

However, the vascular bundles are not affected. The stalks become spongy and can easily be crushed or crimped at lower internodes. The lower internodes become weak and lodge by winds. Pith disintegrates, vascular bundles remain intact. Plants may lodge when pushed sideways or impacted by wind.

(b) *Aetiology*

The pathogen of the disease is *Fusarium moniliforme.* The primary infection is caused by diseased crop debris in soil. The secondary infection is by means of macroconidia and microconidia from diseased plants to healthy plants through field soil and winds.

20.3.12 Bajra/Pearl Millet (*Pennisetum glaucum*)

(a) *Economic Importance*

In Asia and Africa, downy mildews are widespread and highly devastating in pearl millet cropping areas (Williams 1984; Andrews et al. 1985), and worldwide annual grain yield losses range from 20% to 40% (Singh 1995; Hash et al. 1999). If there is repeated cultivation of susceptible cultivar in same field, the losses may be much higher. In southern United States, *Pyricularia* leaf spot is one of the important diseases and also, in India it has emerged as a major disease of grain and fodder pearl millet hybrids (Lukose et al. 2007; Anonymous 2009). The pathogen causes frequent grain yield losses (Timper et al. 2002) and forage losses (Wilson and Gates 1993). Considerable grain yield losses and reduction in fodder quality by rust infection prior to flowering has been recorded (Wilson et al. 1996). Even low disease severity may cause heavy dry matter yield losses in multiple cut forage hybrids (Wilson et al. 1991). The ergot contaminated grains in human consumption or cattle feeds may cause severe toxicity (Mantle 1992). Generally, 5–20% grain yield losses are estimated by smut disease; however, it may be even higher in favourable conditions.

20.3.12.1 *Rhizoctonia* Blight

(a) *Symptomatology*

The infection develops in seed germination and seedling stage. It may cause seed rot, pre- and post-emergence damping-off and collar zone lesions on young seedlings. The canker spots may be seen on grown plants. The colonization to leaf sheath and young leaf tissues appear with dark margin of spots. The midrib may be infected in high severity of disease. The older plants have more number of brown-coloured dead leaves. Also, the pathogen severely damages the root system with discoloured and rapid death of roots.

(b) *Aetiology*

Causal organism: *Rhizoctonia solani* and *Rhizoctonia zeae.* The pathogen survives in the soil as mycelium and dormant sclerotia in left over debris in field. The secondary infection takes place by sclerotia and diseased plant to healthy plants in field.

20.3.12.2 Smut

(a) *Symptomatology*

The symptoms appear during flowering stage, when the infected ovaries of florets convert into structures known as sori (consisting of mass of mycelium). They are larger than normal grain on panicles during grain filling, oval shapes and replace the grains. The initial appearance of these sori is light green coloured. At maturity, the sori become dark brown in colour, filled with teliospores.

(b) *Aetiology*

This disease is caused by *Moesziomyces penicillariae.* The primary infection is caused by infected seeds and crop debris in the field. The secondary infection results from teliospores released and carried by winds and rain water.

20.3.12.3 Ergot

(a) *Symptomatology*

The symptoms of the disease appear during flowering stage. The creamish to pink-coloured "honey dew" secretion is observed from the infected inflorescence of the panicles. Within a period of 10–15 days, this secretion becomes dry, hard and dark brown to black-coloured sclerotia replacing the seeds of the panicles. The sizes of the sclerotia are irregular and larger than the normal seed.

(b) *Aetiology*

This disease is caused by *Claviceps fusiformis.* The primary infection takes place through sclerotia present in the diseased plants in soil by the production of ascospores. The secondary infection is caused by air- and water-borne ascospores infecting stigma of flowers. The relative humidity of more than 80% and temperature range of 20–30 °C are favourable for disease development.

20.3.12.4 *Curvularia* Leaf Spot

(a) *Symptomatology*

The typical minute to small, yellow to brown spots develop on the leaves. These spots enlarge and coalesce to form large lesions. The centre of the disease spots

appears as brown coloured whereas margin remains yellow coloured. The frequency of the disease spots is more common at leaf margins.

(b) *Aetiology*

This disease is caused by *Curvularia penniseti.* The primary infection takes place though infected seeds and conidia on infected plant debris in the field. The secondary infection is caused by conidia of infected plants to healthy plants in crop growing season.

20.3.12.5 *Exserohilum* Leaf Blight

(a) *Symptomatology*

The fungal infection on the leaves is yellow to straw coloured with brown margins. Initially disease spot is dark brown in colour and later turns to light brown. These spots enlarge, coalesce to give blighted appearance. The leaf tips and margins are often blighted by the fungal pathogen.

(b) *Aetiology*

This disease is caused by *Exserohilum rostratum.* The fungal pathogen remains dormant as mycelia and conidia in the crop debris after harvesting. This pathogen may be seed-borne. The secondary infection takes place through conidia spread by winds and rains to healthy plants.

20.3.12.6 Downy Mildew

(a) *Symptomatology*

The visible symptoms on host appear when the fungus gets established as systemic infection. Initially, the symptoms on the leaves appear as chlorotic patches at the base. The higher density of the leaves increases the chlorosis. The diseased leaves of the plants produce higher number of whitish conidia on the lower side of the leaves in cool and humid weather. The highly infected plants are stunted in growth and often unable to produce the panicles. The typical green ear symptoms appear by conversion of reproductive structure (inflorescence) in to leafy structures.

(b) *Aetiology*

This disease is caused by *Sclerospora graminicola.* The primary infection takes place by the surviving oospores and conidia in the infected debris in the soil. The infected seeds may also act as source of infection. The sporangia develop at night with optimum temperature (20 °C) and high relative humidity (70%). The secondary infection results through conidia spread by winds and rain splashes to healthy plants.

20.3.13 Maize (*Zea mays*)

(a) *Economic Importance*

Katinila et al. (1998) and Lyall et al. (2006) reported that *Maize streak virus* is the most important threat to maize cultivation. Several researchers reported complete loss by *Maize streak virus* disease (Wambugu and Wafula 2000; Alegbejo et al. 2002; Lagat et al. 2008). Significant reduction of crop value has been observed by mycotoxins in grains or seeds. In the United States, aflatoxins in maize estimated loss of $225 million per year. In corn, annual yield losses by diseases are estimated from 7% to 20% (Shurtleff 1980).

20.3.13.1 Seedlings and Stalk Rot

(a) *Symptomatology*

Many fungal pathogens are responsible for seedling blight and stalk rot (*F. moniliforme*, *F. oxysporum*, *Penicillium* spp.). In maize, generally two species of fungi (*F. moniliforme* and *F. oxysporum*) cause seedling blights, stalk rots and ear rots. The perfect stage (*Gibberella zeae)* of pathogen (anamorph *Fusarium moniliforme*) is found mostly in cool and humid cropping regions. The infection to ears produces whitish mycelia from terminal end to downwards. Later, pinkish to reddish colouration of the infected kernels may develop. These fungi are known to produce several mycotoxins and cause food poisoning if the infected kernels and seeds are consumed.

(b) *Aetiology*

This disease is caused by *F. moniliforme*, *F. oxysporum* and *Penicillium* spp. The primary infection is caused by mycelia and conidia in infected crop debris in the field. The secondary infection is caused by micro and macro conidia spread by wind, rains, insects and birds.

20.3.13.2 *Bipolaris* Leaf Spot

(a) *Symptomatology*

The typical symptoms develop on the leaves as small lesions with conical to diamond shape. As the lesions develop, they are elongated, coalesce and blight is produced under favourable environmental condition. The infection may be restricted to adjacent veins and hence may appear rectangular in shape at maturity.

(b) *Aetiology*

This disease is caused by *Bipolaris maydis.* The primary infection takes place through infected seed, mycelia and conidia in the leftover crop debris in the field. The secondary infection is caused by conidia from infected to healthy plants in the field.

20.3.13.3 *Curvularia* Leaf Spot

(a) *Symptomatology*

The typical symptoms develop on the leaves by the pathogen. Initially, the small necrotic spots or lesions develop on the leaves and tender parts of host. These spots enlarge to develop chlorotic patches with light coloured halo. The disease is most commonly observed in hot and humid cropping areas and may result in heavy damage to crops.

(b) *Aetiology*

This disease is caused by *Curvularia lunata.* This pathogen is seed-borne, but it can survive as mycelia and conidia in the crop debris in field soil and cause primary infection. The secondary infection is caused by mycelia and conidia from diseased plants to healthy plants in the field.

20.3.14 Makchari/Teosinte (*Zea Mexicana*)

(a) *Economic Importance*

In tropics and semi-tropics, severe economic losses have been reported by corn stunt disease affecting teosinte and maize. In tropics, southern corn leaf blight infects teosinte and sorghum, resulting in heavy yield losses (approx. 70%).

20.3.14.1 Downy Mildew

(a) *Symptomatology*

Two types of typical symptoms are produced by downy mildew pathogen. The foliar symptoms appear as necrotic to chlorotic streaks on upper side of the leaves. The whitish mycelial growth is noticed on upper surface whereas sporangiophores bearing sporangia develops on the lower surface. The infected leaves turn brown, necrotic and dry with disease progress. Also, when the disease extends to tassels, they may be malformed and looks as small and leafy green structures making it sterile.

(b) *Aetiology*

This disease is caused by *Peronosclerospora sorghi.* The primary infection is caused by oospores dormant in the seeds and crop debris in the soil. The secondary infection is caused by sporangia of the diseased plants. The disease spreads by new sporangia carried by winds and rains in high humidity condition.

20.3.14.2 Charcoal Rot

(a) *Symptomatology*

The visible symptoms develop when the crop is at tasselling or next growth stage. The upper leaves of affected plants show drying. Typically, the infected stalks become shredded; the internal tissues like pith gets rotted and strings develop in vascular tissues. The microsclerotia develop in inner portion which are roundish and black in colour and the internal stalk tissue appears grey in colour. The translocation of water and nutrients gets affected by the colonization and disintegration of vascular bundle by the fungus. The lower internode of the stalks becomes weak and cause brakeage and lodging easily.

(b) *Aetiology*

This disease is caused by *Macrophomina phaseolina.* The primary infection is caused by microsclerotia present in the diseased stalks in the field, whereas, secondary infection is caused by spores and mycelia carried by winds and rain water to healthy plants. The crop at hot weather condition and high soil moisture when met with sudden water stress becomes susceptible to disease.

20.3.14.3 Anthracnose Stalk Rot

(a) *Symptomatology*

Initially, small yellow to light brown lesions or spots are formed on the leaf of plant. These yellow-brown spots enlarge gradually with yellow-brown to off-white-coloured lesion with reddish to brown margin. These spots coalesce later to form irregular larger spots. The centre of these spots appears black coloured by the formation of acervuli and setae.

(b) *Aetiology*

This disease is caused by *Colletotrichum graminicola.* The primary infection is caused by infected seeds, spores and mycelia present in crop debris in the field. The secondary infection is caused by slimy mass of spores spread by wind and rains.

20.3.14.4 Southern Leaf Blight

(a) *Symptomatology*

The fungal pathogen often attacks the leaf and sheaths. Initially, the disease spot or lesion is light yellow to brown in colour, oval to typically spindle shape, sometimes linear in shape. However, the margin of the spots becomes distinct brown coloured. These lesions enlarge and coalesce to cause larger irregular leaf blights.

(b) *Aetiology*

This disease is caused by *Cochliobolus heterostrophus.* The primary infection is caused by conidia present in the crop debris. The pathogen can be seed-borne. The secondary infection is spread by lesions from diseased plant and carried to healthy plants by winds and rain splashes.

20.3.15 Chara Sarson/Chinese Cabbage (*Brassica pekinensis*)

(a) *Economic Importance*

The powdery mildew disease causes potential threat for the production of seedlings in Chinese cabbage (Jee et al. 2008). Several workers reported the average yield reduction ranging from 32% to 57% by *Alternaria* blight (Conn and Tewari 1990).

20.3.15.1 *Alternaria* Blight

(a) *Symptomatology*

The minute concentric dark brown spots develop on the leaves which later on turn grey in colour. The spots may be circular to angular in shape with blackish margin. These spots later become papery and brittle with shot-hole appearance. The disease lesions enlarge and extend to stems and petioles. The diseased spots coalesce and give blighted appearance.

(b) *Aetiology*

This disease is caused by *Alternaria raphani* and *A. brassicicola*. The fungus is primarily seed-borne but can also remain dormant in the crop debris left over in the field and causes the primary infection. The secondary infection spreads by spores carried by wind and water.

20.3.15.2 Anthracnose Disease

(a) *Symptomatology*

The minute round- to irregular-shaped disease spots develop on the leaves which later turn grey coloured. The high severity of the disease under favourable weather may cause complete drying of the leaves. These lesions coalesce and enlarge to form larger necrotic patches to give blight appearance and shot-hole appearance on leaves. Wilting may also result in heavy infection.

(b) *Aetiology*

This disease is caused by *Colletotrichum higginsianum.* The primary infection is caused by mycelia present in the crop debris and alternate hosts. The pathogen may be seed-borne. The warm and humid weather favours the disease. The secondary infection is spread by conidia carried by wind and water.

20.3.15.3 Blackleg

(a) *Symptomatology*

The fungal pathogen is most devastating at seedling stage causing damping off of the seedlings. The round- to irregular-shaped spots may develop on leaves and tender stems with dark-coloured margin. In warm and humid weather, pink mycelia develop on the diseased lesions.

(b) *Aetiology*

This disease is caused by *Leptosphaeria maculans*. The primary infection is caused by infected seeds, mycelia and spores in diseased crop debris. The secondary infection of spores spreads by winds and rain splashes. The hot and humid weather favours the rapid disease development.

20.3.15.4 Downy Mildew

(a) *Symptomatology*

The typical mycelial growth develops on underside of leaves. The infected leaves show irregular, yellow to light brown necrotic patches. Later, there is drying of leaves and death of plants.

(b) *Aetiology*

This disease is caused by *Peronospora parasitica.* The primary infection is caused by infected seeds, oospores and mycelia present in the crop debris. The cool and humid weather favours the disease development.

20.4 Conclusion

For the sustainable development of agriculture and textile industry, production of quality forage and fibre crops play a vital role. Occurrence of the diseases and pests affects the availability of quality fodder and fibre. Hence, integrated disease management is the best strategy to minimize the forage and fibre crop losses caused by several diseases. Sowing of disease-free certified seed is very effective way to reduce disease infection at early stages and possible losses. Also, there is need for precise, reliable, rapid and proper diagnostics of seed-borne diseases for effective

management through area wise seed testing laboratories for investigation of particular pathogen(s) in seed lots. Transportation of the infected seeds results in long-term dispersal and may establish the pathogen and diseases in the new areas. Thus, it is very important to diagnose the seed lots for the presence or absence of pathogens.

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Part VII

Seed-Borne Diseases: Human and Animal Health

Seed-Borne Diseases: Its Impact on Human and Animal Health

21

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Abstract

Healthy seed plays an important role in ensuring productivity and profitability of crops. Contamination of seed with various toxins adversely impacts human and animal health. Fungal species produce toxic metabolites called mycotoxins that contaminate staple foods and feeds. They represent an unavoidable problem due to their presence in globally consumed cereals. Mycotoxin may be hepatotoxins, nephrotoxins, and neurotoxins. Majority of mycotoxins cause suppression of the immune system, and some are carcinogenic in nature and adversely affect human and animal health and reduce livestock production. Stricter control of mycotoxins has been envisioned worldwide. While human beings are adapting to cope with environmental changes, such as food scarcity, decreased food quality, mycotoxin regulations, crop production, and climate change, fungal species are also adapting, and increased cases of mycotoxins-induced adverse health are likely to occur in the future. It is of vital importance for different countries to set mycotoxin limits and regulations taking into account both scientific and socio-economic factors. The first limits for mycotoxins were set in the late 1960s for the aflatoxins, and by the end of 2003, several countries had developed specific limits for mycotoxins in foodstuffs and feedstuffs, and the number continues to grow. To ensure food security, we need a way to balance global mycotoxin standards which are realizable, considering limitations of producers and designing strategies to reduce mycotoxin exposure based on sound research.

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21.1 Introduction

Seed-borne diseases are diseases of plants that are transmitted by seed. In some cases the seed transmission is insignificant as compared to the population density of the pathogen that exists in soil or on weed species. In other instances, the disease spreads primarily through seed. Diseases of plants are caused primarily by bacteria, fungi, and viruses. Fungi comprise the largest group of pathogens, but the majority of seed-specific diseases are caused by bacteria or viruses. This is due to the fact that bacteria and viruses are able to enter and then travel through the veins of the plant easily, they reach the vascular system, and finally they develop in the embryos of seeds.

Generally, fungi remain confined to the outer layers of the plant; they initiate infection by means of air-borne spores and then proliferate by infecting cells of the outer layers. The possibility of the fungi entering the plant vascular system is low, and they cause seed infection mostly when they are able to either "travel" all the way to seed on the outside of the plant or release spores that fall on the seed. The fungal spores are either on the outer surface of the seed or in the layers of the seed coat. Spores on the seed surface are likely to either dry up and die or slough off with the seed coat during seed germination, thereby failing to cause disease in the next generation. Besides the crop losses caused by seed-borne pathogens, the contamination of food and feed crops by the mycotoxins produced by seed-borne fungi takes heavy toll of human and animal health globally. This problem is further exasperated by lack or lax implementation of laws to regulate the threshold limit of mycotoxins in different crops especially in developing countries. Globalization of trade has led to free movement of food grains across boundaries; therefore, it is of utmost importance to have globally set standards to limit or reduce the level of mycotoxins below the threshold limit to protect human and animal health (Table 21.1).

21.2 Seed Health

Seed health is very important as it is a universal truth that seed is the basic unit of crop production and, therefore, food production. When seeds are used for sowing, seed-borne pathogens may cause disease or death of plants resulting in crop loss (IRRI 2009). Seed health refers to the presence or absence of disease-causing organisms such as fungi, nematodes, bacteria, viruses, and insects and to the status of seeds in a seed lot. Seed status is also affected by the presence of non-disease-causing contaminants in a particular seed lot. These include contaminants like weed seeds that compete with the target seed for nutrients, other seeds, plant parts other than the target seeds, soil particles, and insect eggs that can overwinter and can degrade the quality of seed lot.

Table 21.1 Important seed-borne fungal diseases of major crops in India

Crops	Diseases	Pathogen
Wheat	Loose smut	*Ustilago segetum* var. *tritici*
	Karnal bunt	*Tilletia indica*
	Flag smut	*Urocystis agropyri*
Chickpea	*Ascochyta* blight	*Ascochyta rabiei*
	Wilt	*Fusarium oxysporum* f.sp. *ciceri*
Crucifers	Gray and black leaf spot	*Alternaria brassicae*
		A. brassicicola
Rice	Bunt	*Tilletia barclayana*
	False smut[a]	*Ustilaginoidea virens*
	Blast	*Magnaporthe grisea*
	Stackburn	*Alternaria padwickii*
Cotton	Anthracnose	*Colletotrichum indicum*
	Wilt	*F. oxysporum* f.sp. *vasinfectum*
Maize	Black kernel rot	*Botryodiplodia theobromae*
	Cob rot	*Fusarium moniliforme*
	Southern leaf blight	*Drechslera maydis*
Pearl millet	Downy mildew	*Sclerospora graminicola*
	Smut	*Tolyposporium penicillariae*
Sorghum	Anthracnose	*Colletotrichum graminicola*
	Kernel or grain smut	*Sphacelotheca sorghi*
	Downy mildew	*Peronosclerospora sorghi*

[a]Not seed-borne but affect grain/seed

21.3 Crop Losses Caused by Seed-Borne Diseases

In India, maize, groundnut, rice, cotton seeds, and millets can be categorized as high-risk commodities (Huchchannanavar and Balol 2011).

21.3.1 Maize

The major seed-borne pathogens of maize are *Diplodia* spp. (*D. macrospora*, *D. maydis*, *D. frumenti*), dry or white ear rot, stalk rot; *Bipolaris* (*Helminthosporium*) *maydis*, southern leaf spot or blight; *Bipolaris turcica*, northern leaf spot; *F. graminearum*, charred ear mold; and *F. moniliforme*, cob rot. Almost half of the world's maize is grown in North and Central America. Two third of losses due to diseases are caused by seed-borne pathogens (Cramer 1967), and of these *Diplodia* and *F. graminearum* account for about 25% and *Drechslera* spp. for almost 20% of the total loss due to diseases.

Maize contamination with fumonisin has been reported from many parts of the world with reported levels greater than 100 mg/Kg in a few areas. Factors such as geographical region, season, and the conditions under which crop is grown, harvested, and stored determine fumonisin contamination of agricultural produce.

Grain grown in tropical and subtropical regions is more prone to fumonisin contamination due to the relatively long warm growing season. There are reports of high levels of fumonisin contamination of corn from Tanzania, South Africa, the United States, and China (Gamanya and Sibanda 2001; Fandohan et al. 2005; Shephard et al. 2007; Kimanya et al. 2008). Consumption of contaminated maize has been associated with an elevated risk of human esophageal cancer in the Transkei region in South Africa and China (Williams et al. 2010). In 2004, the largest mycotoxin-poisoning epidemic occurred in Kenya (CDC 2004; Lewis et al. 2005). Aflatoxin poisoning was associated with eating home-grown maize stored under damp conditions (Lewis et al. 2005). Eastern and central provinces of Kenya continue to suffer from acute aflatoxin poisoning (Ngindu et al. 1982; CDC 2004; Lewis et al. 2005). In 2004 there was an outbreak of aflatoxin poisoning; aflatoxin B1 concentrations in maize were as high as 4400 ppb, which is 220 times greater than the 20-ppb regulatory limit. More than seven districts were affected by the outbreak that resulted in 317 cases of patients and 125 deaths (Lewis et al. 2005). Other minor outbreaks were reported in 2005 and 2004 with about 30 and 9 deaths, respectively (Ministry of Agriculture 2006, 2007). The maize implicated in the 2004 aflatoxicosis outbreak was harvested in February during off-season, with early rains.

Towards the end of 1974, there was an epidemic of an unusual liver disease in circumscribed rural areas of two northwestern states of India, viz., Rajasthan and Gujarat. This outbreak was investigated separately by teams of scientists from the National Institute of Nutrition, Hyderabad, and the All India Institute of Medical Sciences, New Delhi. The results have strongly suggested that the disease outbreak was the result of consumption of aflatoxin-contaminated maize (Srikantia 1982).

In 1974, in Western India, 200 villages were reported to have been affected by an outbreak of hepatitis due to aflatoxicoses in Banswada and Panchamahals districts of Rajasthan and Gujarat, respectively, that caused 106 deaths. The outbreak lasted for 2 months and was confined to tribal population whose main food was maize. Analysis of *Aspergillus flavus*-contaminated (6.25–15.6 ppm) maize samples showed that affected people might have consumed between 2000 and 6000 μg Kg-1 (ppb) aflatoxins daily over a period of 1 month (Krishnamachari et al. 1975a, b). Development of ascites, edema of the lower limbs, portal hypertensions, and higher mortality rate were the symptoms of the diseases. An independent study on the same outbreak also confirmed that the aflatoxins were the major cause of this disease (Bhat and Krishnamachari 1978). Another outbreak of toxic hepatitis in India in 1974 affecting both humans and dogs was also reported (Tandon et al. 1977).

21.3.2 Groundnut

In India, it was reported that over a decade, the export of groundnut extractions has declined from 550 metric tons to 265 metric tons valued at US $ 32.5 million due to the presence of aflatoxins (Codex 1998). Each importing country has prescribed the standards for permissible level for aflatoxin in groundnut. Permissible level for

aflatoxin in groundnut is 30 ppb per Kg in India. As per Indian Council of Medical Research (ICMR), New Delhi, 21% of groundnut and maize samples in India are unfit for human consumption because of aflatoxin contamination. In order to protect the international trade, in the 29th meeting, codex committee on food additives and contaminants (CCFAC) proposed the maximum draft level of 15 microgram per Kg for total aflatoxin in peanuts that are to be further processed. If this level is applied, 37% of our groundnut samples are rejected. In Andhra Pradesh groundnut samples contain 15–19% excess aflatoxin than the permissible level (Ghewande 1997; Reddy et al. 2011).

Egal et al. (2005) have reported that 90% of children in West Africa (Benin and Togo) are exposed to aflatoxins due to consumption of contaminated maize and groundnuts which leads to a measurable impairment of child growth. Outbreaks of acute aflatoxicosis from contaminated groundnut in humans have been reported in India, Malaysia, Thailand, and Kenya (CAST 2003). In India, the first report of aflatoxins in humans was from 150 villages of Western India in 1974 where 397 persons were affected and 108 persons died (Krishnamachari et al. 1975b). Groundnut samples from Ethiopia indicated heavy aflatoxin contamination at levels much higher than any international acceptable standards, e.g., FAO and WHO acceptable limit being 15 μg/Kg (Guchi 2015).

As a result of these stringent regulations, the import of groundnut meal in the EC has declined from 0.91 million metric tons in 1979–1980 to 0.4 million metric tons in 1989–1990.

A case study in India of an outbreak of aflatoxicosis in a poultry farm revealed that an 18-day exposure of poultry to the contaminated feed containing 600 μg/Kg AFB1, contributed mainly from groundnut cake, resulted in a loss of about 10% of the initial investment (Prathapkumar et al. 1997). The major loss was observed to be due to a drop in egg production followed by mortality in birds and additional expenditure on the protein source.

India is the second largest producer of groundnut and is faced with difficulties in accessing these markets due to high level of aflatoxin on Indian groundnut that is deemed unfit for human consumption. In accordance with Agmark standards, the level of aflatoxin permitted in India is 30 ppb. Owing to high food safety and animal and plant health concerns, several countries over the last few years have issued many SPS (sanitary and phytosanitary) notifications against India. This has led to shrinkage in exports of groundnut oil to many developed and developing countries (Enamul et al. 2015).

More than 200,000 broiler chickens died in 1994 in Ranga Reddy district of Andhra Pradesh, India, because the groundnut cake in feeds was contaminated with aflatoxin (Anonymous 2002). Similarly, aflatoxicosis led to heavy mortality in chicks in Chittoor district of Andhra Pradesh state in India (Char et al. 1982).

21.3.3 Millets

It is very well documented that the presence of ergot alkaloids in pearl millet is known since time immemorial. Pearl millet grains are generally not considered as good substrates for mycotoxin contamination when compared to groundnut and maize. But the earlier studies indicated that pearl millet grains are subjected to mold contamination with dominance of *Aspergillus flavus* and *A. parasiticus* (Mathur et al. 1973; Konde et al. 1980; Girisham et al. 1985; Singh and Singh 2004; Chintapalli et al. 2006; Reddy et al. 2006; Raghavender et al. 2007).

Veno-occlusive disease outbreak occurred in India. The people had consumed millets, as staples, inadvertently contaminated with toxic seeds of the weed, *Crotalaria*. A similar outbreak was also reported in Afghanistan. The affected people suffered from pain in epigastrium and ascites that lead to liver damage. *Crotalaria* and millets often grow in the same environment, and as a result its seeds often get mixed with the food grain during harvesting (Bhat 2008). There have been reports of consumption of ergot in pearl millet and other grains that caused vomiting, acute nausea, and dizziness in India and East African countries and gangrene, a classic ergot poisoning symptom in Ethiopia (Bhat and Vasanthi 2003).

Mycotoxin-producing *Penicillium*, *Fusarium*, and *Aspergillus* have been isolated from sorghum and pearl millet in India (Waliyar et al. 2004), while ergot (*Claviceps fusiformis*) has been reported as a common field disease in pearl millet (Hill and Waller 1988). In India, infestation of pearl millet by a parasitic fungus, *Claviceps purpurea*, has caused ergotism, which is characterized by symptoms of somnolence, nausea, vomiting, and giddiness (Patel et al. 1958; Krishnamachari and Bhat 1976).

21.4 Mycotoxins

A diverse group of fungi produce toxic metabolites called mycotoxins. The metabolites contaminate agricultural produce either during harvest or during storage. Varying levels of mortality and morbidity are exhibited by different species including humans, poultry, swine, fish, etc. upon exposure to these harmful substances (Zychowski et al. 2013). Society is faced with a very important issue of maintaining a secure global food and feed supply. Natural contaminants, especially mycotoxins, pose a challenge since they are found in a wide range of crops and differ significantly in chemical structure. The symptoms induced also differ in humans and in animals upon exposure (Kendra and Dyer 2007) and can encompass a broad range of effects including neurotoxicity, carcinogenicity, and developmental toxicity (Kolpin et al. 2014).

In addition to public health, the demand of food products in international markets is limited or reduced due to the presence of dangerous substances (Frenich et al. 2014). Therefore, international organizations like Food and Drug Administration (FDA, Food and Drugs Administration 1995) and European Commission (European Commission 2006) have established maximum residue limits (MRLs) for mycotoxins. Many countries have enacted legislation based on scientific risk assessment to

reduce exposure to mycotoxins, which allows small amounts of mycotoxins in foods or feeds, which are lower than what is confirmed not to affect human and animal health (Henson and Caswell 1999).

Mycotoxins pose a risk to human and animal health by contaminating food and cause significant economic losses. As per Rodrigues et al. (2011), these losses are supported by all participants along the chain of animal producers and those who handle and distribute grain, processors of crops, and also consumers in society. It is difficult to confirm that the disease is mycotoxicosis and to establish the identity of etiological agents in a given veterinary or human health problem even when mycotoxins are detected. Although it is hard to define, there is conclusive proof from animal models and human epidemiological data to conclude that mycotoxins pose threat to human and animal health. In a nutshell, due to lack of suitable diagnostic criteria and reliable laboratory tests, the mycotoxicosis remains a diagnostically daunting disease (Zain 2011).

Humans and livestock production are impacted economically by mycotoxins, and this impact can be assessed by a number of criteria based on human and animal life loss, healthcare and veterinary care costs, economic loss in livestock production, forage crops and feeds, regulatory costs, and research cost aimed at ameliorating the mycotoxin impact and severity. It has been difficult to develop formulas for worldwide economic impact assessment, and, therefore, in most cases economic impact assessment has been done based on a single aspect of mycotoxin exposure or contamination (Hussein and Brasel 2001). Table 21.2 shows the incidence of mycotoxin contamination in 4327 samples of different grains (maize, oats, wheat,

Table 21.2 Incidence of mycotoxin contamination of samples of grain (maize, oats, wheat, barley) in the world according to Biomin (2011), during the years 2009–2010 and 2012–2013, respectively

	AFB1 (%)		ZEN (%)		DON (%)		FBs (%)		OTA (%)	
Region/years	09–10	12–13	09–10	12–13	09–10	12–13	09–10	12–13	09–10	12–13
North America	21	26	14	9	50	33	27	55	21	2
South America	13	12	28	39	21	33	76	76	16	2
Africa	50	67	8	26	17	67	58	78	12	56
Europe										
Northern	Nd	32	25	34	71	87	Nd	86	Nd	40
Central	19	29	41	26	64	66	51	36	20	28
Southern	33	55	14	18	36	50	56	71	41	46
Asia										
North	15	14	63	57	83	79	51	47	25	15
South	88	59	14	26	22	36	56	57	49	55
Southeast	71	59	37	49	34	79	55	47	28	15
Middle east	37	30	Nd	19	11	46	67	78	50	31
Oceania	6	4	26	19	49	24	12	16	11	13

AFB aflatoxins, *ZEN* zearalenone, *DON* deoxynivalenol, *FB* fumonisin, *OTA* ochratoxin A; n = 4327 (2009/10) and n = 4200 (2012/2013)

barley) around the world. Taking into account the data from Table 21.2, it can be affirmed that the occurrence of AFB1 found in the Middle East, Asia, and African countries is very high and worrying. The incidence of ZEN in Northern Asia; DON in North America, North Europe, and China; FBs in South America, Africa, Europe, Asia, and the Middle East; but also OTA in the Middle East were present in more than 50% of the samples analyzed (Biomin 2011).

21.4.1 Aflatoxins

The discovery of aflatoxins came about after studies on the cause of the death of 100,000 of turkey poults ("Turkey X disease"), ducklings, and chicks in England in 1960 that caused a loss to the tune of several hundred thousand dollars (Allcroft and Carnaghan 1962). The study established that mortality was due to contamination of feed. It was a shipment of Brazilian peanut meal used as poultry feed produced by Old Cake Mills, Ltd. in London. This meal, called Rosetti meal (from the name of the ship in which it was imported), was toxic and carcinogenic and contaminated with the common fungus, *Aspergillus flavus*. A group in England and the Netherlands extracted and isolated the active principles from *A. flavus* cultures (Nesbitt et al. 1962; Van der Zijden et al. 1962). The active principle was chemically identified by a research group in the USA (Asao et al. 1963). The toxin was named aflatoxin where the "a" is from *Aspergillus* and the "fl" from *flavus*.

21.4.2 Negative Effects of Mycotoxins on Humans

Human beings are most susceptible to aflatoxicosis illness in developing countries. This is because in these countries security blankets at preharvest and postharvest level in crops are not as strict as in developed countries. Humans are most commonly exposed to the effects of aflatoxin in three ways (Radmila et al. 2013):

- Ingestion of food of vegetable origin (mainly corn and peanuts) contaminated with aflatoxin (AFB1).
- Ingestion of contaminated milk and dairy products, including cheese and powdered milk (AFM1).
- Ingestion of aflatoxin residues present in meat and meat products, as well as in eggs that are present in a lower degree than in the previous two modes.

The adverse effects of aflatoxins in humans and animals have been categorized in two general forms as follows.

21.4.2.1 Acute Aflatoxicosis

Consumption of moderate to high levels of aflatoxins causes acute aflatoxicosis. Specific acute episodes of disease include hemorrhage and acute liver damage called severe hepatotoxicity with a case fatality rate of approximately 25%. There is

edema, absorption and/or metabolism of nutrients, and alteration in digestion. The early symptoms of hepatotoxicity due to aflatoxicosis can comprise malaise, anorexia, and low-grade fever. Acute high-level exposure can progress to potentially lethal hepatitis which is marked by vomiting, abdominal pain, jaundice, fulminant hepatic failure, and death (Walderhaug 1992; Cullen and Newberne 1994; Strosnider et al. 2006).

21.4.2.2 Chronic Aflatoxicosis

It results from ingestion of low to moderate levels of aflatoxins. The effects are usually subclinical and difficult to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome (Walderhaug 1992). *Fusarium* species have been implicated in several outbreaks of mycotoxicoses in human. Cereal grains contaminated with *Fusarium sporotrichioides* and *F. poae* were implicated in alimentary toxic aleukia in Russia from 1932 to 1947. Symptoms included mucous membrane hyperemia, esophageal pain, laryngitis, asphyxiation, gastroenteritis, and vertigo (Lewis et al. 2005). Aflatoxicosis is a toxic hepatitis leading to jaundice and, in severe cases, death. Repetitive incidents of this nature have occurred in Kenya during 1981, 2001, 2004, and 2005, India, and Malaysia (Shephard 2004; Lewis et al. 2005). AFB1 has been extensively linked to human primary liver cancer in which it acts synergistically with HBV infection and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (Group 1 carcinogen) (IARC 1993). This combination represents a heavy cancer burden in developing countries. A recent comparison of the estimated population risk between Kenya and France highlighted the greater burden that can be placed on developing countries (Shephard 2006). Aflatoxins have been found in tissues of children suffering from kwashiorkor and Reye's syndrome and were thought to be a contributing factor to these diseases. Reye's syndrome, which is characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral edema (Blunden et al. 1991). Aflatoxin has long been linked to kwashiorkor, a disease usually considered a form of protein energy malnutrition, although some characteristics of the disease are known to be among the pathological effects caused by aflatoxins in animals. The degree of toxicity will depend on the toxin(s) present, dosage, duration of exposure, and a variety of other factors. Species, age, hormonal status, nutrition, and concurrent disease are considered the most important (Bryden 2007; Wild 2007). The gut microflora may also modify mycotoxin toxicity (Swanson et al. 1988; Annison and Bryden 1998; Eriksen et al. 2002).

21.4.3 Negative Effects of Mycotoxins on Non-ruminants

Consumption of aflatoxins results in similar effects in all animals; however, the susceptibility varies with species, age, and individual capacity to tolerate toxicity. Acute aflatoxicosis is characterized by depression, anorexia, weight loss, disease, gastrointestinal bleeding, liver damage, and pulmonary edema symptoms. Acute

hepatic injury leads to coagulopathy, increased capillary fragility, hemorrhage, and prolonged clotting times. Blood pigments may appear in the urine, and mucous membranes are icteric. Gross changes in the liver are caused by centrilobular congestion and hemorrhage and fatty changes of surviving hepatocytes. The animal dies within hours or a few days. Exposure to moderate levels of aflatoxins for a long time leads to a decline in feed consumption and production, viz., growth and production of eggs and milk (Lizárraga-Paulín et al. 2011).

21.4.3.1 Poultry

Broiler-type chickens are more resistant to aflatoxin toxicity than other poultry species (Arafa et al. 1981). AFB1 in the poultry industry is referred to as "the silent murderer" because evident clinical symptoms are not induced due to its chronic consumption at levels below 20 ppb; however, it reduces the absorption of food and causes immunosuppression. Many studies have demonstrated that chicken performance is negatively affected due to mycotoxins, for example, reduction in body weight and increase in liver and kidney weights after feeding a high level (3.5 mg/Kg of feed) of an AF mixture (i.e., 79% AFB1, 16% AFG1, 4% AFB2, and 1% AFG2) to broilers (Smith et al. 1992). Aflatoxins cause increase in blood urea N and decrease in serum levels of total protein, albumin, triglycerides, and phosphorus.

21.4.3.2 Pigs

Swine are very sensitive to mycotoxins. The swine immune response to AF has been inconsistent. Feeding mixed AF at levels ranging from 0.4 to 0.8 mg/Kg of feed to levels as high as 500 mg/Kg of feed did not alter swine humoral immune response. The immunosuppression caused by AF (140 or 280 μg/Kg of feed) only occurs at the cellular and not the humoral level, and there was inhibition of DNA synthesis in porcine lymphocytes when AFB1 was added to the medium at various levels (0.1–10,000 ng/ml of medium) (Pang and Pan 1994). Mycotoxin zearalenone (ZEN) negatively affects swine reproductive function (Diekman and Green 1992). Pigs have been shown to draw the toxic forms of ZEN back from the circulating glucuronide conjugate. Due to this reason, the estrogenic effects of ZEN have been strong and prolonged in pigs. Study in Hungarian farms showed feeding ZEN-contaminated feed to sexually mature gilts results in swelling of the vulva and mammary glands and occasional vaginal and rectal prolapses (Glavitis and Vanyi 1995).

21.4.3.3 Horses

Asquith (1991) has reviewed the history of mycotoxicosis and poisoning in equine. In a case study, mature horses consuming AFB1-contaminated feed (58.4 μg/Kg) were jaundiced and anorexic before death. Postmortem examinations showed enlargement of livers, damage to the kidney, and bile duct hyperplasia lesions. In other cases, equine aflatoxicosis has been marked by lameness, depression, and death. Postmortem examinations revealed subcutaneous and enteric hemorrhage, enlarged necrotic livers, enlarged kidneys, and hepatic, nephritic, and myocardial lesions.

21.4.3.4 Dogs and Cats

Mycotoxins have severe effects on companion animals and can lead to death. As with other species, the primary target organ of ochratoxin A (OTA) in dogs and cats is the kidney. In a study, pacing and vomiting were observed at an OTA dose of 0.2 mg/Kg in dogs. At doses between 0.2 and 3.0 mg/Kg, symptoms of intoxication in dogs included anorexia, polydipsia, polyuria, anxiety, prostration, and death. The necropsy findings included epithelial degeneration (proximal tubules), mucohemorrhagic enteritis (cecum, colon, and rectum), and necrosis of the lymphoid tissues (spleen, tonsil, thymus, and peripheral lymph nodes) (Bird 2000).

21.4.4 Negative Effects of Mycotoxins on Ruminants

Ruminants including cattle, sheep, goats, and deer are less known for their sensitivity to the negative effects of mycotoxins as compared to non-ruminants. However, consumption of mycotoxin-contaminated feed for extended periods of time by ruminants results in alteration in production (milk, beef, or wool), reproduction, and growth (Hussein and Brasel 2001).

21.4.4.1 Cattle

The first case of poisoning in cattle by groundnut was reported in 1961. Calves (3–9 months old) had eaten for at least 6 weeks a compounded aflatoxin-contaminated groundnut. There were areas of fibrosis with biliary proliferation and veno-occlusive disease in the liver of animals. In other cases, an increase was found in connective tissue too, and degeneration of centrilobular hepatic cells was described. Icterus, weight loss, and death were reported (Newberne and Butler 1969). Milch cattle are mainly affected because of the infection mechanism they suffer. Milch cattle affected by chronic aflatoxicosis caused by the prolonged feeding of concentrate feed mixtures containing contaminated groundnut cake having aflatoxin B1 (110 μg/Kg groundnut cake at the time of sampling), B2, G1, and G2 were subjected to pathological, hematological, and plasma enzymatic studies. Clinical and necropsy observations on the liver included proliferation of connective tissue along portal triads leaving small group of hepatocytes intact. Liver function tests showed liver damage in three of the four affected animals studied (Vaid et al. 1981) (Table 21.3).

21.4.5 Economic Impact of Mycotoxins

There are multiple criteria for assessing the economic impact of mycotoxins on humans and on animals. Considerations include loss of human and animal life, healthcare and veterinary care costs, loss of livestock production, loss of forage crops and feeds, regulatory costs, and research cost focusing on relieving the impact and severity of the mycotoxin problem. Formulas for worldwide economic impact have been difficult to develop, and therefore, most reports on economic impact are on a single aspect of mycotoxin exposure or contamination (Zain 2011).

Table 21.3 Toxic effects of selected mycotoxins (Bryden 2012)

Mycotoxin	Clinical signs
Aflatoxins	Liver damage; reduced productivity; inferior egg shell and carcass quality; increased susceptibility to disease
Cyclopiazonic acid	Liver, kidney, and gastrointestinal tract damage; weight loss, weakness, egg shell problems; inappetence, diarrhea, dehydration, depression, opisthotonos, and convulsions
Zearalenone	Swollen, reddened vulva, vaginal prolapse, and sometimes rectal prolapse in pigs; suckling piglets may show enlargement of vulvae; fertility problems
Deoxynivalenol	Decreased feed intake and weight gain in pigs with DON at >2 mg/Kg feed; vomiting and feed refusal at very high concentrations of DON (>20 mg/Kg diet)
Other trichothecenes	More toxic than DON; reduced feed intake; emesis, skin and gastrointestinal irritation; neurotoxicity; abnormal offspring; increased sensitivity to disease; hemorrhaging
Ochratoxin A	Mainly affects proximal tubules of the kidneys in pigs and poultry; kidneys are grossly enlarged and pale; fatty livers in poultry
Fumonisin B1	Equine leukoencephalomalacia (ELEM); porcine pulmonary edema (PPE); hepatocarcinogenic in rats; and possibly esophageal cancer in humans
Ergot alkaloids	Nervous system disorders; tremors; convulsions; diarrhea; necrosis of the extremities (gangrene); reduced feed intake; abortion, stillbirth, and agalactia (cessation of milk production); blackening of the comb, toes, and beak in poultry. In high environmental temperatures, necrosis of extremities may not be evident, but animals may experience hyperthermia

In India, the economy is affected heavily by mycotoxins. A study carried out in the Bihar region from 1985 to 1987 (Ranjan and Sinha 1991) found nearly 51% of the 387 samples tested to be contaminated with molds. Out of 139 samples containing AF, 133 had levels above 20 μg/Kg. In another study (Phillips et al. 1996), levels as high as 3700 μg/Kg of AF were reported in groundnut meal used for dairy cattle. Twenty-one of 28 dairy feed samples from farms in and around Ludhiana and Punjab were reported to be contaminated with AFB1 at levels ranging from 50 to 400 μg/Kg (Dhand et al. 1998). India lost 10 million dollars export within a decade due to groundnut contamination with mycotoxins (Vasanthi and Bhat 1998) (Table 21.4).

21.4.6 Bioterrorism

Mycotoxins can be used as chemical warfare agents (Ciegler 1986). There is considerable evidence that Iraq had bioweapons program during the 1980s and Iraqi scientists developed aflatoxins. They cultured toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* and extracted aflatoxins to produce over 2300 liters of concentrated toxin. The majority of this aflatoxin was used to fill warheads; the remainder was stockpiled (Zilinskas 1997; Stone 2001). The choice of aflatoxins for chemical warfare seems curious because the induction of liver cancer is "hardly a knockout punch on the battlefield" (Stone 2002). However, terrorists seek to elicit

Table 21.4 Some of the highest values of aflatoxin contamination in the rejected lots of different food commodities of India, based on the Rapid Alert System for Food and Feed (RASFF 2011)

S. No.	Commodity	Maximum contamination levels (μg/Kg)		Date of case
		B1	Total	
1	Groundnut	118.0	281.0	15/04/2011
2	Stone-ground corn meal	410	430	08/08/2008
3	Mixed snacks	184.07	188	12/12/2007
4	Corn meal in retail pack	47	51	06/02/2009
5	Unpolished basmati rice	46.2	50.7	07/12/2007
6	Ground turmeric and whole nutmeg	700	1200	18/10/2010
7	Ground and broken nutmeg	230	249	03/09/2008
8	Nutmeg powder	79	97	21/01/2010
9	Ground nutmeg	50	58.2	27/04/2010
10	Turmeric powder	48	53	24/12/2010
11	Turmeric powder	48	52	29/04/2009
12	Chili powder	47.2	48.7	05/11/2010
13	Organic ground nutmeg	41.1	–	28/05/2010
14	Crushed chilies	38	40	27/10/2008
15	Clove powder	26.4	27.4	17/02/2009
16	Chili powder	24	25	31/08/2010
17	Dried red chili	23	25	17/12/2010
18	Dry whole chilies	20	21	24/11/2010
19	Ginger	13	24	19/04/2011

the repugnance and emotional response by the use of chemical and biological weapons. Furthermore, with their use against ethnic groups such as the Kurds, the long-term physical and psychological results could be devastating (Bennett and Klich 2003). Finally, some experts think that selection of aflatoxin was due to the reason that it was the "pet" toxin of an influential Iraqi scientist (Stone 2002).

21.5 Legislation for Safety Level of Mycotoxins in Human Food, Animal Feed, and Animal Feed Ingredients

As per the Food and Agriculture Organization (FAO) of the United Nations (2014), hundreds of million people, especially women and children in the developing world, are affected by micronutrient deficiencies. The number of childrens worldwide that are at risk of vitamin A deficiency and goiter or are iron deficient has been estimated to be more than 200 million. Rural diets in many countries are not diverse, and, therefore, it is important to have good food sources that can provide all essential nutrients in people's diets. All regulations are primarily made on the basis of known toxic effects in animals or humans. To prepare correct exposure assessment, reliability of data on the presence of mycotoxins in different commodities and data on food intake is very important to prepare exposure assessment. During the past decades in many countries in spite of difficulties, mycotoxin regulations have been

established, and newer regulations are still being prepared. Figure 21.1 depicts the countries that have some kind of legislation for foodstuffs and animal feedstuffs (Anater et al. 2016).

21.6 Global Trends of Mycotoxin Regulation

Food security, as defined by the WHO at the World Food Summit of 1996, exists when all people, at all times, have physical and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life. However, no singular food is fully innocuous, and people have dealt with unsafe foods since inception. It is desirable that our diets can supply needed nutrients with minimum amount of potentially toxic contaminants. Naturally occurring mycotoxins are unavoidable, especially in developing countries; therefore, achieving this ideal balance is tough. Despite the efforts made by the FAO, the WHO, and agencies in other countries in setting regulations to limit the amounts of mycotoxins in foods, many countries still lack appropriate guidelines to limit these toxins (particularly in Africa and Latin America). The decision of which mycotoxins to regulate and at what levels is left to the scientists and health officials of each country, although most of these limits are based on globally set standards. In today's world, food is traded freely among several nations, and new global regulations must be established to safeguard consumers. Several countries have taken initial steps regarding global food protection from mycotoxins. It involves the documentation and evaluation of regulatory procedures for feed ingredients. For example, a report

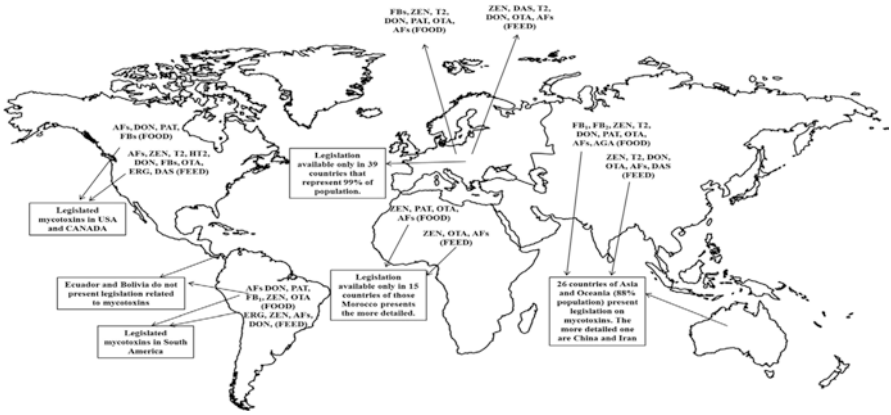

Fig. 21.1 Countries that have some kind of legislation for foodstuffs and animal feedstuffs. In accordance to FDA Mycotoxin Regulatory Guidance (FDA 2011a, 2011b) and CODEX, there is the action level for AFBs, and there are advisory levels for two mycotoxins that may be present in human food and animal feed ingredients: DON (vomitoxin) and FBs. The action level for AFBs present in human food is 20 ppb and animal feed and animal feed ingredients 20–300 ppb; and advisory levels for DON present in human food are 1 ppm and animal feed and animal feed ingredients 5–10 ppm and for FBs present in human food are 2–4 ppm

entitled "Comparison of Regulatory Management of Authorized Ingredients, Approval Processes, and Risk-Assessment Procedures for Feed Ingredients" was written on behalf of the International Feed Industry Federation for Brazil, China, Canada, European Union, Japan, South Africa, and the United States (Smedley 2013; Cardona et al. 2014).

21.7 Mitigation for Mycotoxin

Mycotoxins pose a serious risk to human health, especially in developing countries where by circumscribing mechanisms of biochemical detoxification, the detrimental effects of these food-borne toxins are exacerbated by poverty and malnutrition. Even in cases where it is known that moldy grain is not safe for human health, such grain may be fed to livestock, decreasing animal productivity and the food supply and increasing poverty (Wu et al. 2005). Epidemiological evidence for mycotoxins as causative factors of various diseases is still lacking, although it is recognized that aflatoxin is a causative factor for primary liver cancer. In developing countries, mycotoxins place a significant constraint in attempts to improve human health; therefore, exposure to these compounds should be urgently addressed as a food safety issue. Sustainable development goals aim at reducing and eliminating hunger and malnutrition by 2030. This includes provision of safe food which is free from contaminants including mycotoxins.

21.7.1 Mitigation Strategies

An integrated understanding of crop biology, agronomy, fungal ecology, methods of harvesting, storage conditions, processing of feed, and detoxification strategies is required to minimize incidences and the impact of mycotoxins. Bryden (2009) has reviewed this topic comprehensively. A number of approaches can be taken to minimize mycotoxin contamination in the food/feed chain. These involve steps to prevent fungal growth and therefore mycotoxin formation, strategies to reduce/eliminate mycotoxins from contaminated feedstuffs, or diverting contaminated products to low-risk uses including animal feeds.

The following general approaches are applicable to the mitigation of mycotoxin contamination of the human food chain (Bryden 2009) and animal feed chain:

- Genetic modification of fungi and crops.
- Agronomic and biological control measures.
- Climate modelling to predict mycotoxin risk.
- Storage management.
- Food processing.
- Detoxification.
- Integrated mycotoxin management.
- Human intervention.

Feed may be contaminated with mycotoxin at any point in the feed production chain, and integrated mycotoxin management is an approach that applies Hazard Analysis Critical Control Points (HACCP) to reduce contamination throughout the chain. The applications of this approach reduce mycotoxin contamination in all sectors of the chain and have the added advantage of increasing production efficiency (Lopez-Garcia 2001; Aldred and Magan 2004). A number of HACCP programs (Aldred and Magan 2004; Lopez-Garcia et al. 2008) have been developed for aflatoxins in maize, animal feeds, copra and coconut, and groundnuts and pistachio nuts and for ochratoxin A in coffee. The establishment of a HACCP program should evolve in conjunction with other complementary approaches to agricultural production, namely, good agricultural practice, good manufacturing practice, and good hygiene and storage practice. Therefore, preventive measures comprise good agronomic practices to improve plant vigor, the judicious use of insecticides and fungicides to reduce infestation by insects and fungi, irrigation to avoid drought stress and harvesting at maturity, and, more recently, plant breeding programs to enhance genetic resistance to fungal attack (Bryden 2009).

21.7.1.1 Feed Storage and Processing

It is important to prevent productions of mycotoxins in stored feeds to control mycotoxicoses in livestock and poultry (Smith and Henderson 1991). Good feed management practices along with methods that modify the fungal environment have to be followed. Several compounds are available that inhibit mold growth in feedstuffs. Organic acids, especially propionic acid, are used in many commercial antifungal agents used in the stockfeed industry and give excellent protection (Hamilton 1985).

21.7.1.2 Feed Additives

In the animal feed industry, various well-established practices including feed additives to alleviate nutrient deficiencies, increasing product pigmentation, improving pellet quality, breaking down of anti-nutritive factors, and adsorption of toxicants and toxins are being used. Different substances have been investigated as potential mycotoxin-binding agents (Galvano et al. 2001; Huwig et al. 2001; Jouany 2007; Oguz 2011), including lucerne, synthetic cation or anion exchange zeolites, bentonite, bleaching clays, spent canola oil, and hydrated sodium calcium aluminosilicate (HSCAS). Successful binding agents prevent absorption of the toxin by the intestine of the animal from the contaminated feed. Many studies have examined the effectiveness of HSCAS to reduce the toxicity of a variety of mycotoxins in livestock and poultry. HSCAS adsorbs aflatoxins, forming a very stable complex with the toxin and hence reducing its bioavailability but is less effective with other mycotoxins (Phillips 1999; Phillips et al. 2002). Investigations on the use of HSCAS are being carried out to reduce human exposure to aflatoxin (Phillips et al. 2008). Dawson et al. (2001) and Jouany (2007) have reviewed the use of a yeast cell wall-derived glucomanin prepared from *Saccharomyces cerevisiae* which has been shown in vitro to efficiently adsorb aflatoxin, zearalenone, and fumonisins.

Preharvest steps taken to minimize the risk of mold contamination and mycotoxin production should be based on the use of certified seed, or it is to be ensured that seed is free from fungal, bacterial, or viral infection. Irrigate the crop to avoid drought stress; sow seed early so that crop matures early. Crop residues are to be removed and minimum or zero tillage is practiced; insect, mammal, bird, and virus pests are to be controlled; rotate crops; avoid nutrient stress (FAO 2007). Action to be taken during harvest to reduce the risk of mold contamination and mycotoxin production should be based on harvest as quickly as possible; avoid field drying; transport the crop to the homestead as quickly as possible. The crop is to be dried on aboveground raised platforms; bundles of stover should also be kept on platforms to dry and not left to lie on the soil (FAO 2004, 2007).

Postharvest actions to reduce the risk of mold contamination and mycotoxin production should be based on drying the crop on a polythene sheet, tarpaulin, or empty sacks laid on the ground or on a concrete plinth; unthreshed crop can be laid on a platform or in a ventilated crib to dry; cobs can also be tied in pairs and suspended from a vertical frame to dry. Solar dryer can be used to dry the crop in wet or humid conditions. Crop should be threshed, shelled, and winnowed carefully so that grain is not broken or damaged. Grain should not be beaten with sticks as this causes lots of damages which lead the grains to develop mold. Grains are to be stored in a suitable container which is raised above ground level and should be water proof and must have protectant to prevent insect and rodents damage. External storage structures must have a good roof with suitable overhang. Sacks must be kept on a platform raised above the floor and inspected regularly, and flour and feed must only be stored for short periods (FAO 2007). According Guan et al. (2009), the discovery of the mycotoxin-transforming microorganisms may provide new opportunities for managing mycotoxin problems (Table 21.5).

21.8 Need and Scope for Managing Seed-Borne Diseases

India is signatory to the World Trade Organization (WTO). Seed trade now comes under the purview of international agreements of the WTO. With the removal of quantitative restrictions on imports from April 1, 2001, in India, seed-borne diseases, seed health testing, and seed certification procedures have become very important.

21.9 Seed Certification

Seed certification for a crop embodies legal norms to ensure genetic identity, physical purity, germinability, and freedom from seed-transmitted weeds and pathogens. International Seed Testing Association (ISTA) and Association of Official Seed Certifying Agencies (AOSCA) have developed minimum seed certification standards.

Table 21.5 Permissible limits of mycotoxins in food for humans, animal feed, and animal feed ingredients (Anater et al. 2016)

Mycotoxins (μg/Kg)	Food typology			
	1	2	3	4
Aflatoxins (B1, B2, G1, G2)				
South America	5	20	0–30	10
EUA	20	20	Nd	Nd
Canada	Nd	Nd	Nd	20
Africa	11,079	42,278	10	44,105
Europe	4–10	4	4	10
Asia	15–20	50	20	10–1000
Oceania	5	5	5	5
Ochratoxin A				
South America	18,537	18,537	2	Nd
UAE	5	5	3	Nd
Africa	Nd	Nd	0	300
Europe	3	3	Nd	Nd
Deoxynivalenol				
South America	3000	1000	200	Nd
UAE	1000	Nd	Nd	Nd
Canada	2000	Nd	Nd	1000–5000
Fumonisin				
South America	Nd	2000	200	Nd
Zearalenone				
South America	200	400	20–200	Nd

1 = cereal and cereal products; 2 = corn; 3 = cereal-based foods for baby food and infant formula; 4 = feed to livestock production and pet animals, *nd* not defined

Essentially, the certification procedures ensure the genetic purity and quality of seed production in the field, during harvest, processing, storage, and finally inspection in the market. Seeds distributed to farmers are guaranteed to have genetic and physical purity and germination capacity. Certification for seed-borne pathogens is followed only as and when required depending on the impact of the pathogen on yields.

21.10 Status of Seed Health Certification in India

Seed improvement program in India began with the setting up of the NSC Ltd. during the 1960s. The State Farms Corporation of India and the Tarai Development Corporation were set up in 1963 and 1969, respectively. The Seed Review Team (1968) and National Commission on Agriculture (1971) made several recommendations for the expansion of the seed sector. Indian Seeds Act (1966) was enacted to maintain quality of seeds during production and distribution stages. In 1972, Central Seed Certification Board (CSCB) was set up with a view to advise the Central and State Governments on all matters relating to seed certification. Seed industry has

achieved phenomenal growth since then. Nineteen seed certification agencies have been set up in the country. The area under seed certification, which was a few hundred hectares in the early stage of seed certification, has increased to more than 5,00,000 hectares. The certification procedure involves four steps, viz., (1) verification of seed source for raising seed crop; (2) crop inspection to verify conformity to seed standards; (3) seed testing; and (4) postharvest supervision. The Seeds Act (1966) and the New Policy on Seed Development (1988) aim at promotion and regulation of the seed industry. With globalization and economic liberalization, several new opportunities as well as challenges have come up. The National Seeds Policy that came into existence in 2002 provides renewed quality assurance mechanisms.

21.11 Plant Quarantine

The terms frequently used in plant quarantine are hazard, risk, and safeguards. Hazard is the danger that a specified pathogen is known to present to the agriculture of the importing country, should the pathogen gain entry on imported items and subsequently become established. Risk is the chance that a hazardous organism will enter and become established. Safeguards are action taken to reduce the risk of introducing hazardous organism.

The term quarantine is derived from the Latin word "quarantum," meaning 40. It refers to the 40-day period of detention of ships arriving from countries with bubonic plague and cholera in the Middle Ages. Plant quarantine promulgated by a government or group of governments restricts entry of plant, plant products, soil, culture of living organisms, and packing materials to protect agriculture and the environment from avoidable damage by hazardous organisms. The importance of plant quarantine has increased due to the increase in exchange of seeds or grains for consumption along with better means of transportation.

21.11.1 Plant Quarantine in India

The awareness on transboundary movement of pests and of quarantine measures in India started in the early twentieth century when the Indian Government in 1906 ordered compulsory fumigation of imported cotton bales to prevent introduction of Mexican cotton boll weevil (*Anthonomus grandis*). The quarantine law was introduced for the first time in the country by the then Governor General of India on February 3, 1914. It was known as Destructive Insects and Pests (DIP) Act. A gazette notification entitled Rules for Regulating the Import of Plants, etc. into India was published in 1936, and custom authorities were entrusted to enforce the plant quarantine regulations. Though the DIP Act was revised several times over the years, yet it needs to be periodically reviewed to meet the fast-changing global scenario (Kheterpal 2004). In addition, the government of India has approved three national institutes, viz., the National Bureau of Plant Genetic Resources, New

Delhi, for agricultural and horticultural crops; Forest Research Institute, Dehradun, for forest plants; and Botanical Survey of India, Kolkata, for all other plants of economic interest for ensuring pest-free exchange of plant material. As per Plant Quarantine (Regulation of Import into India) Order (2003), a total of 60 entry points including 34 seaports, 12 airports, and 14 land custom stations are notified points of entry for import of plants and plant material. Besides 60 Inland Container Depot/ Container Freight Station, 11 Foreign Post Offices have also been notified for the entry of plants/plant material under the PQ Order, 2003.

21.12 Conclusion

Seeds play an important role in pathogen transmission and disease spread. Contaminations of seed with mycotoxins have implications for human and animal health. Fungi cause human illness in different ways, but toxic secondary metabolites produced by saprophytic species are an important health hazard. Based on animal models and human epidemiological data, it has been proven that mycotoxins pose a serious risk to human and animal health, albeit one that is hard to pin down. There is a need to come up with strategies to cope with this problem because mycotoxins are unavoidable. Most of the mycotoxin outbreaks have taken place in developed nations are also at risk exposure due to imports of contaminated food. Despite best efforts by regulatory agencies to control permissible levels of mycotoxins, sometimes introduction of contaminated imported foods or food ingredients can take place because of difficulties and different ground realities faced by countries.

Monitoring of traditional mycotoxins in foods has become easier due to availability of better analytical capabilities; however, the overall management of food toxins may be complicated due to emergence of a myriad of new mycotoxins. Besides, developing countries may not have access to new technologies due to economic constraints, and this may delay implementation of global regulations. Fungi are adapting to changing global environment, and increased mycotoxin contamination of commodities in uncommon places is likely to take place due to occurrence of favorable environmental conditions for growth of fungi. Food scarcity will complicate mycotoxin management even more. There is a need of continued research on understanding the effects and modes of mycotoxin action in various species so as to be able to manage them effectively.

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Part VIII

Management of Seed-Borne Pathogens/ Diseases

Use of Biocontrol Agents for the Management of Seed-Borne Diseases

22

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Abstract

Seeds are the most critical input in crop production system, and 90% of all the worlds' food crops are raised from seeds. Seeds are distributed at large scale in market and are now responsible more than ever for the dissemination of plant pathogens across vast distances. Seed-borne pathogens are continuously imposing a serious threat to crop production and are responsible for the reemergence of various diseases through the introduction of particular diseases into new areas. Seed-borne pathogens are of particular importance because strategies used for their management are insufficient, especially with the availability of limited and outdated chemicals. Various strategies have been employed to manage seed-borne diseases including cultural, chemical, regulatory, and biological methods. In the past few decades, chemicals are widely used for seed treatment as a potent approach toward disease control, and commencement of systemic fungicides added further possibilities to it. However, rising concern about their negative impact on the environment and human health minimizes their use. Application of agriculturally important microorganisms for disease management is a safer alternative to conventional management practices which have severely affected the environment and agroecosystem. Application of beneficial microbial antagonists to seed for managing seed and soil-borne pathogens is model delivery systems as it brings in the microbial inoculum to the rhizosphere.

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22.1 Introduction

Plethora of plant pathogens infect the crop plants and reduce the quantity and quality of crop production. Various plant pathogens particularly seed-borne pathogens are critical for the quality seed production as they infect the seeds and may remain in the seed for long time. Seeds are the most critical input in crop production system, and 90% of all the worlds' food crops are raised from seeds. Furthermore, seeds are distributed at large scale in market and are now responsible more than ever for the dissemination of plant pathogens across vast distances. Seed-borne pathogens are continuously imposing a serious threat to crop production and are responsible for the reemergence of various diseases through the introduction of particular diseases into new areas. Seed-borne pathogens are of particular importance because strategies used for their management are insufficient, especially with the availability of limited and outdated chemicals. In the current crop production system, two methods are now being applied for increasing crop productivity, i.e., introduction of high-yielding varieties and avoidance of crop disease incidence. However, seed-borne diseases are responsible for about 10% losses in major crops in India, thereby minimizing the benefits of using high-yielding variety. Planting an infected seed may lead to a widespread distribution of disease within healthy crop and increased number of primary infection sites from where the diseases can disseminate.

Various strategies have been employed to manage seed-borne diseases including cultural, chemical, regulatory, and biological methods. There are certain regulation and rules which control the cultivation of seed production and seed distribution between national and international trades. These legislations are forced to prevent the spread of infected planting materials and pathogens into country or states. Such regulations are enforced by means of quarantine and inspection of seed production sites and warehouses and occasionally by eradicating certain host plants. Plant quarantine measures include two basic principles of plant disease management, i.e., exclusion and eradication. In quarantine law, plant materials and germplasm are regulated, and the entry of infected seeds and microorganisms is checked by treatment and inspection, or the movement of the host is restricted or banned. Since 1870, growth of quarantine regulations has escalated, and currently each country has its own regulatory bodies and laws. In 1914, the Government of India passed the Destructive Insect and Pests Act (DIP Act) to prevent the movement of infected seed materials and pathogens.

Adjustment of crop production practices accordingly is an age old practice with the primary aim of reduction of yield losses caused by diseases and pests. Precise adjustment in cultural practices modifies the surrounding environment that is favorable for host but not favorable for the pathogens. Cultural practices are vital part of sustainable agriculture and are considered as crucial backup procedures for disease management. These practices include seed production in dry climate, adjustment of sowing time, managing isolation distance for seed plots, inspection of seed plots, adjustment of harvesting time, and drying and cleaning of seeds. Various physical methods are also used for managing seed-borne diseases including hot water treatment, hot air treatment, solar heat treatment, etc. Hot water treatment was first used for ornamental plants in Scotland, and since then it is widely accepted method for controlling various seed-borne pathogens. In addition to this, treatment with hot air

is also practiced and is less injurious to seeds. This method is easy to perform but less effective in comparison to hot water treatment. This method is generally used for sugarcane setts treatment for managing ratoon stunting disease on commercial scale.

A large number of seed-borne pathogens are carried and introduced by the seeds. Seed treatment is the application of protectants and toxic chemical to seed to prevent the movement of pathogens in or on the seeds. It also allows the seed to overcome early infection caused by soil-borne pathogens. In the past few decades, chemicals were widely used for seed treatment as a potent approach toward disease control, and commencement of systemic fungicides added further possibilities to it. However, rising concern about their negative impact on the environment and human health minimizes their use. Application of agriculturally important microorganisms for disease management is a safer alternative to conventional management practices which have severely affected the environment and agroecosystem (Abhilash et al. 2016). Beneficial microorganisms including plant growth-promoting rhizobacteria (PGPRs), plant growth-promoting fungi (PGPFs), biological control agents (BCAs), and endophytes play a crucial role in sustainable disease management. In addition to the disease management, these microorganisms provide plant growth promotion and mitigation of abiotic stresses. In the last decades, various genera of PGPRs and PGPFs have been screened, characterized, and identified, and their application has been boosted manifolds. Bacterial genera including *Bacillus*, *Pseudomonas*, *Serratia*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Burkholderia*, and *Alcaligenes* have been reported as potential biocontrol agents, biofertilizers, and biostimulants (Keswani et al. 2014, 2016a, b; Bisen et al. 2015; Keswani 2015; Singh 2014). Furthermore, various fungal genera have also been investigated for their beneficial traits. Beneficial effects of *Trichoderma* spp. on plant disease management and growth promotion are frequently studied and are currently being commercialized as biopesticide (Singh 2006; Keswani et al. 2013; Bisen et al. 2016). More than 60% of the globally marketed biopesticides are based on *Trichoderma* formulation (Keswani et al. 2013; Singh 2014). About 250 products are commercially available for field applications in India alone (Singh et al. 2012).

22.2 Seed Inoculants for Control of Seed-Borne Diseases

Despite the aims for which beneficial microorganisms are applied to the crops, they must be applied in a way that optimize their efficacy in the field environment. Beneficial microorganisms have been delivered by many techniques as liquids and dry formulations. Seed treatment with beneficial microorganisms is a very old practice. Legume seed inoculation with nitrogen-fixing bacteria has a long history and enhances the legume production worldwide (Graham and Vance 2003). However, despite encouraging results of legume seed inoculation and in vitro demonstration of the efficacy of other beneficial microorganisms, there are still very few commercially available microbial seed inoculants. Seed treatment with broad-spectrum fungicides is often essential to escape seedling establishment failure caused by various seed or soil-borne phytopathogens. Application of beneficial microbial antagonists to seed for managing seed and soil-borne pathogens is a model delivery system as it

brings in the microbial inoculum to the rhizosphere. Wide range of both fungal and bacterial antagonists have been commercially exploited for this purpose (Nelson 2004; Berg 2009), but their application as seed treatment is very limited.

Various beneficial antagonistic microorganisms including both fungi and bacteria are now being employed as seed inoculants for managing seed-borne diseases in wide range of crops (Table 22.1). *Trichoderma* spp. have been extensively evaluated as a seed inoculant for managing seed-borne pathogen such *Ustilago segetum* var.

Table 22.1 Biocontrol agents used against various seed-borne pathogens

Crops	Diseases	Biocontrol agents	References
Cereals	*Ustilago segetum* var. *tritici*	*Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, and *Gliocladium virens*	Singh and Maheshwari (2001)
	Tilletia caries	*Pseudomonas chlororaphis* MA 342	Johnsson et al. (1998)
	Ustilago nuda	*P. chlororaphis* MA 342	Johnsson et al. (1998)
	Tilletia tritici	*P. chororaphidis* MA 342, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *T. harzianum*	Borgen and Davanlou (2001)
	Cochliobolus sativus	*Chaetomium globosum*	Aggarwal et al. (2004)
	Septoria tritici	*T. harzianum*, *T. koningii*, *Bacillus megaterium*	Perelló et al. (2009), Kildea et al. (2008), and Cordo et al. (2007)
	Mycosphaerella graminicola	*Bacillus megaterium*	Kildea et al. (2008)
	Urocystis agropyri	*Azotobacter* spp., *Gluconacetobacter* spp., *Bacillus thuringiensis*	Wadhwa et al. (2011) and Tao et al. (2014)
	Helminthosporium oryzae	*T. viride*, *Pseudomonas fluorescens*	Arumugam et al. (2013)
	Pyricularia oryzae	*T. viride*, *P. fluorescens*, *Pseudomonas syringae* pv. *syringae*	Arumugam et al. (2013) and Smith and Métraux (1991)
	Drechslera graminea	*Trichoderma* spp.	Koch et al. (2006)
	Drechslera maydis	*Streptomyces* spp. (DAUFPE 11470, DAUFPE 14632)	Bressan (2003)
	Drechslera teres	*P. chororaphidis* MA 342	Hökeberg et al. (1997)
	Fusarium culmorum	*Chaetomium* sp., *Idriella bolleyi*, *Gliocladium roseum*	Knudsen et al. (1995)
	Bipolaris sorokiniana	*Chaetomium* sp., *Idriella bolleyi*, and *Gliocladium roseum*	Knudsen et al. (1995)
	Magnaporthe grisea	*Stenotrophomonas maltophilia*, *F. solani* KS-F14, *Achromobacter xylosoxidans*, *Streptomyces globisporus* JK-1	Etesami and Alikhani (2016), Chern et al. (2014), Joe et al. (2012), and Li et al. (2011)

(continued)

Table 22.1 (continued)

Crops	Diseases	Biocontrol agents	References
	Xanthomonas oryzae pv. *oryzae*	*Bacillus subtilis* GBO3, *Bacillus pumilus* SE34, *B. pumilus* T4, *P. fluorescens* PTB 9, *P. fluorescens*, *Lysobacter antibioticus*, *T. harzianum*, *T. hamatum*, *T. virens*, endophytic *Streptomyces* spp., *Pseudomonas* spp., *P. putida* V14i	Udayashankar et al. (2011), Velusamy et al. (2006), Vidhyasekaran et al. (2001), Ji et al. (2008), Rangarajan et al. (2003), and Kavitha (1999)
	Pseudomonas syringae pv. *syringae*	*Pantoea agglomerans*	Braun-Kiewnick et al. (2000)
	Xanthomonas oryzae pv. *oryzicola*	*Bacillus amyloliquefaciens*	Zhang et al. (2011)
Pulses	*Botrytis* spp.	*T. harzianum*, *T. viride*, *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Penicillium griseofulvum*, *Trichoderma hamatum*, *Penicillium chrysogenum*, *P. brevicompactum*, *Cladosporium cladosporioides*, *Chaetomium globosum*, *Pseudomonas aeruginosa*, *Epicoccum nigrum*, *Penicillium citrinum*, *Penicillium chrysogenum*	Mukherjee and Haware (1993), Huang and Erickson (2002), Jackson et al. (1994), Szandala and Backhouse (2001), Freeman et al. (2004), Sreevidya et al. (2015), and Jackson et al. (1994)
	Xanthomonas axonopodis pv. *phaseoli*	*Rahnella aquatilis*, *Pseudomonas* spp., *Bacillus* spp., *Pseudomonas aeruginosa*, *Rhodococcus fascians*, *Bacillus cereus*	Sallam (2011), Giorgio et al. (2016), and Spago et al. (2014)
	Ascochyta rabiei	*Chaetomium globosum*, *T. viride*, *Acremonium implicatum*, *T. harzianum*	Rajakumar et al. (2005) and Benzohra et al. (2011)
	Phoma medicaginis	*Bacillus licheniformis*	Slimene et al. (2015)
	Macrophomina phaseolina	*T. harzianum*, *G. virens*, *Paecilomyces lilacinus*, *Streptomyces* sp., *Rhizobium meliloti*, *B.subtilis* BN1, *P. fluorescens*	Hussain et al. (1990) and Arora et al. (2001)
Vegetables	*Alternaria solani*	*Pseudomonas fluorescens* (*Pf1* and *Py15*), *Bacillus subtilis* (*Bs16*)	Latha et al. (2009)
	Fusarium species	*Burkholderia cepacia*	Recep et al. (2009)
	Colletotrichum lindemuthianum	*T. viride*, *T. harzianum*, *Gliocladium virens*, *P. fluorescens*	Amin et al. (2014)
	Pyrenochaeta lycopersici	Strain K61 of *Streptomyces griseoviridis* (*Mycostop*®)	Minuto et al. (2006)
	Fusarium oxysporum f. sp. *lycopersici*	*Penicillium oxalicum*	De Cal et al. (1997)
	F. oxysporum f. sp. *radicis-lycopersici*	*T. harzianum*, *T. koningii*	Bourbos et al. (1997)
	Botrytis aclada	*Ulocladium atrum*	Yohalem et al. (2004)

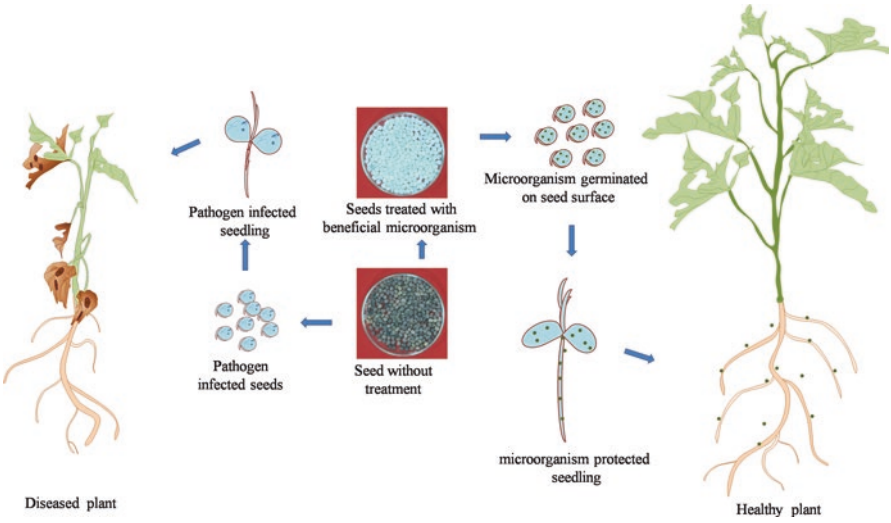

Fig. 22.1 Effect of seed treatment with agriculturally microorganisms on plant health

tritici, *Septoria tritici*, *Pyricularia oryzae*, *Xanthomonas oryzae* pv. *oryzae*, *Botrytis* spp., *Macrophomina phaseolina*, *Rhizoctonia solani*, etc. *Trichoderma* spp. have also been reported to promote plant growth through various additional mechanisms such as enhancing root proliferation and induction of systemic resistance (Fig. 22.1). When used as seed treatment, *Trichoderma* spp. have been shown to induce improvements in seed and subsequent crop performance (Harman 2006). A range of *Trichoderma* formulation-based biopesticide products are commercially available in the market, but to date they have been infrequently used as a seed treatment despite the successful demonstration under greenhouse and field conditions (Copping and Menn 2000; Nagaraju et al. 2012).

22.3 Seed Biopriming: A New Approach for Seed Inoculation with Beneficial Microorganisms

With the advancement of seed priming, intensive researches have been conducted, and it is now being commonly used for seed inoculation for better crop establishment and yield. Seed priming provides model environment to bioagents for colonization of the seed. "Soaking the seeds in solution containing desired microorganism followed by re-drying of the seeds that result into start of germination process except the radicle emergence is seed biopriming" (McDonald 1999). According to the Abuamsha et al. (2011) "Soaking the seeds in the bacterial suspension for precalculated period of time to allow the bacterial imbibition into the seed is known as biopriming." Seed soaking in bioagent suspension resulted in activation of physiological processes in the seed. But emergence of plumule and radical is prevented until the seeds are sown. Seed biopriming with PGPRs has been performed in

Table 22.2 Representative list of biocontrol agents used for seed biopriming in different crops against various pathogens

Crop	Bioagent	Pathogen	References
Carrot	*Clonostachys rosea*	*Alternaria dauci* and *A. radicina*	Jensen et al. (2004)
Maize	*T. harzianum*	*Fusarium verticillioides*	Nayaka et al. (2010)
Sunflower	*P. fluorescens*	*Alternaria helianthi*	Rao et al. (2009)
Pearl millet	*P. fluorescens*	*Sclerospora graminicola*	Raj et al. (2004)

various crops including sweet corn (Callan et al. 1991), carrot (Jensen et al. 2004), and tomato (Harman and Taylor 1988). Seed biopriming has been reported to facilitate the survival of bioagents in/on seed surface, thus providing better plant growth and yield (Bisen et al. 2015; Singh et al. 2016; Singh 2016) (Table 22.2).

22.4 Research Requirement and Opportunities

Although extensive researches have been done on various applications of agriculturally important microorganisms, a very few are used as seed inoculants. Challenges are high in improving the efficacy of seed inoculants to perform their functions under varied environmental conditions, but taking into consideration the potential benefits to be achieved from this approach, further research is urgently needed.

22.4.1 Novel Formulation, Biomass Production, and Seed Treatment Processes

Development of commercially viable microorganisms for seed treatments is supported by mass production and formulation technology. Production of a large and effective biomass of particular microbial agent used in commercial formulation is influenced by the production method, medium constituents, and production parameters. Extensive research is needed to standardize the production procedure (Hynes and Boyetchko 2006). In addition to advances in microbial biomass production, growth in commercialization of biological seed treatments is largely dependent on the development of novel formulation technologies. In recent years, research efforts in formulation of microorganisms have intensified that will support and strengthen the delivery of microorganisms through seed. While most of these researches are still very sensitive to commercialization, some new approaches such as microencapsulation are being explored. Application of these technologies in combination is required to overcome the incompatibility of seed inoculants. For example, a bacterium *Pseudomonas putida*, which was dried with osmoprotectants, remained viable in encapsulated form and applied to maize seeds (Manzanera et al. 2004). It is uncertain that this multistep process could be progressed economically, but it presents a useful proof of concept.

Certainly, it is clear that the current seed treatment practices including rapid drying at high temperature are challenged with viability of microbes on seed. Thus, seed treatment companies must consider the other production option to ensure the product quality. Hartley et al. (2012) reported that the slow drying of seed often showed improved survival of microorganism on seed. Moenne-Loccoz et al. (1999) reported that the drying (20 h and 3 h) of treated seed enhanced the survival of *P. fluorescens*, but this prolonged drying period is not fit for current intensive farming. Collaborative efforts between seed companies and experts in microbial formulation would address these challenges and hurdles.

22.4.2 Biology of Seed and Rhizosphere

Information regarding to the fate of inoculants in the short-lived and dynamic spermosphere is very little known (Nelson 2004). Microbial communities present on seed surface is proliferated and stimulated by seed exudates released during the germination. Better understanding of characteristics of seed-associated microorganisms and seed inoculants will provide greater insight for developing various procedures for effective seed inoculation. The fate of microbial inoculation of seed is not only dependent on the formulation technologies and storage but also on its ability to multiply and colonize spermosphere. The interaction between seed inoculants with soil components is not investigated very well, but it greatly influences the establishment of inoculants in soil and root zone. Knox et al. (2004) reported the role of soil nematode in root colonization of wheat seed inoculant *P. fluorescens*. Few researches have been undertaken to investigate the interaction between seed inoculants and rhizosphere. With the advancement of new molecular technologies, study of seed inoculants activity will provide new insight and could be used for the development of high-performing inoculants and formulation.

22.4.3 Microbial Consortia

Prior art is loaded with the evidence that co-inoculation with consortia improved the plant growth and reduced the disease incidence in comparison to single microorganism. Application of multiple BCAs with differing modes of action may help to overcome the hurdles and variability frequently observed in field trials. Synergistic effects of microbial consortium have been proved to provide better elicitation of resistance responses, plant growth, and abiotic stress tolerance (Jain et al. 2015a, b). There may be increased cost associated with production of multiple inoculant strains, but where clear benefits can be shown, this cost is justified. Compatibility between the co-inoculants and their modes of action is essential.

22.4.4 Plant Endophytes

There is an increasing interest in exploring the role of microbial endophytes in improving crop performance during biotic and abiotic stresses and application as seed inoculants. Endophytes generally live at least part of their life cycle within the host and provide protection from pest and diseases (Ray et al. 2016; Hastuti et al. 2012). Various reports have suggested the augmented induction of defense response in crop plants against wide range of phytopathogens. Joe et al. (2012) have reported that the seed inoculation of rice with endophytic bacterium *Achromobacter xylosoxidans* significantly increased the rice yield by stimulating plant defense against rice blast disease. To date limited interest has been shown in utilization of beneficial endophytic microorganisms as seed inoculants. Lots of significant opportunities lie in terms of the selection and use of effective inoculants from within the plants, and these opportunities must be addressed.

22.5 Conclusion

Crop production is the outcome of interaction between various factors. Seeds decide the fate of efficacy of all other factors. It is a most vital input in agriculture. Sowing of quality seeds contributes largely to the higher yield and income to the farmers. Seed-borne diseases are major threat to the crop production in modern agriculture as they are highly circulated in global market. Since the introduction of systemic fungicides and other seed treatment chemicals, the market of chemical seed treatment is rising more than ever. However, concern over harmful effects of chemicals on environment and human health is pushing the growers to use eco-friendly practices for seed treatment to manage seed-borne diseases. Biocontrol agents offer a safe alternative to the harmful chemicals. Various BCAs have been utilized as seed inoculants and their effect on crop performance under biotic stresses are evaluated. Despite the potential advantages offered by BCAs in laboratory conditions, a very little has changed regarding to the numbers of biological seed treatment products in the market. Better understanding on the use of beneficial microorganism as seed inoculants will rely on collaborative research and experiments between institutions and industry. A greater research incorporating multidisciplinary approaches such as rhizosphere and seed biology, seed physiology, microbe's physiology, formulation technology, and chemistry (adjuvants and carrier) will lead to the development of successful biological seed inoculants.

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Chemical Management of Seed-Borne Diseases: Achievements and Future Challenges

23

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Abstract

Seed is a basic and most important input for agriculture and high-quality seed is also required in international seed trading. There are many seed-borne diseases and pathogens that have negative impact on seed health and its quality. Seed-borne inoculums are the main source of primary infection leading to disease development. The disease gets established in the field wherever the infected seed is used and causes severe yield losses. Paul Neergaard is considered as father of seed pathology who has contributed a lot in the development of seed pathology. Seed-borne pathogenic microorganisms mainly fungi, viruses and bacteria are greatly affecting seed quality and cause diseases that affect seedling production in the nurseries. Management strategies for the control of various seed-borne diseases are mainly based on chemical seed treatment. Studies have been carried out with a view to find out the best way to use chemicals in seed production. Such studies are very few, and hence, there is a need to conduct further research so that new chemicals could be incorporated into seed-borne disease management

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programmes. We need to have additional ways to control diseases that are seed-borne, and chemical pesticides are effective against a wide spectrum of diseases. This chapter provides a brief review of seed-borne pathogen problems that affect seeds and discusses established and potential control practices by using chemicals like fungicides, insecticides or defence activators and further gives the future perspectives where focus should be given with respect to seed pathology and management of seed-borne disease.

23.1 Introduction

Seed occupies only a small niche in the overall agricultural economy, yet the value of this commodity is greatly amplified by the fact that high-quality seed is the basis for future agricultural production. The losses caused to the agricultural economy by seed-borne diseases are present in many forms. Losses may be immediate due to reduced germination, poor seedling vigour, abnormal seedlings and other damage to the crop at any stage of growth from seedling to harvest to storage. Losses also can occur in the first crop produced by the infected seed lot or, as is often the case, when the pathogens are able to survive in the soil and on crop debris and weed hosts, providing reservoirs of inoculums to attack succeeding susceptible crops. Through agriculture's history, seed-borne diseases have been one of the most severe problems in cultivation (Brent and Hollomon 2007a, b). Many plant pathogens present in soil constantly attack the plants. Three main types of pathogens, viz. bacteria, fungi and viruses, cause various diseases of plants. Fungi comprise the largest group of pathogens, but bacteria and viruses cause a large number of seed-specific diseases. This is due to the fact that bacteria and viruses are more adept at entering and then travelling through the veins of the plant, a phenomenon known as 'systemic infection', and from the vascular system may move into the developing embryos of seed. A lot of diseases can be transmitted both by seeds and through soil (Table 23.1). If conditions become favourable for infection, plants develop diseases. Seed treatment is therapeutic when it kills bacteria or fungi that infect embryos, cotyledons or endosperms under the seed coat, eradicative when it kills spores of fungi that contaminate seed surfaces and protective when it prevents penetration of pathogenic fungi into seedling stems (Epners 1964; Brent and Hollomon 2007a, b). Plant pathogens present in, on and around planted seed are killed by chemical treatments. Certified seed is usually given treatment necessary for the control of certain diseases. Seed treatment can be either physical or chemical. Physical treatments include hot-water treatment, solar-heat treatment (loose smut of wheat) and the like. Chemical treatments consist of use of fungicides and bactericides. Fungicides such as oxathins (carboxin, DMOC) are used to kill embryo infecting smuts of cereal grain. Most eradicative and protective chemicals have a wide range of fungicidal activity. Organic compounds now widely used as protective and eradicative seed treatments include thiram, chloraneb, dichlone, dexon and captan (Miller and Bramlett 1979).

Table 23.1 List of some important seed-borne diseases and their causal agents

Disease	Causal organism
Karnal bunt of wheat and triticale	*Tilletia indica*
Bunt of paddy	*Neovossia horrida*
Ergot of pearl millet	*Claviceps fusiformis*
Ergot of sorghum	*Sphacelia sorghi*
Ergot of triticale	*Claviceps purpurea*
Loose smut of wheat	*Ustilago tritici*
I.ate blight of potato	*Phytophthora infestans*
Dry rot of potato	*Fusarium caeruleum*
Charcoal rot of potato	*Macrophomina phaseolina*
Wet rot of potato	*Sclerotium rolfsii*
Common scab of potato	*Streptomyces scabies*
Black scurf of potato	*Rhizoctonia solani*
Basal rot of onion	*Fusarium oxysporum* f. sp. *cepae*
Soft rot of onion	*Erwinia carotovora*
Black rot of sweet potato	*Ceratostomella fimbriata*
Scurf of sweet potato	*Monilochaetes infuscans*
Wilt of sweet potato	*Fusarium oxysporum* f. sp. *batatas*
Bacterial blight in chickpea	*Xanthomonas campestris* pv. *cassie*
Anthracnose of lentil	*Colletotrichum truncatum*
Alternaria blight of chickpea	*Alternaria alternata*
Grey mould (*Botrytis*) of lentil	*Botrytis cinerea*
White mould	*Sclerotinia sclerotiorum*
Stemphylium blight (chickpea, lentil)	*Stemphylium botryosum*
Bright yellow mottle of chickpea	*Alfalfa mosaic virus* (AMV)
Bright mosaic of field pea	*Bean yellow mosaic virus* (BYMV)

The International Seed Testing Association (ISTA) has established seed testing procedures. ISTA came into being in 1924, with the plan of developing and publishing standard procedures in the field of seed testing, and has laboratories in over 70 countries. ISTA has internationally agreed rules for seed sampling and testing, accrediting laboratories, promoting research and providing international seed analysis certificates and training and dissemination of seed science knowledge and technology to facilitate seed trading nationally and internationally. The regulation of seed-borne diseases must, thus, be based on a systematic preventive approach in the production of seeds combined with monitoring of the occurrence of diseases. Economically important seed-borne fungal diseases of vegetables and flowers include *Alternaria zinniae* on Zinnia (*Zinnia elegans* L.) and other flowers (Wu and Chou 1995), *Fusarium oxysporum* f. sp. *callistephi* on China aster (*Callistephus chinensis* (L.) Nees.) (Orlicz-Luthardt 1998) (Table 23.1).

23.2 Types of Seed-Borne Pathogens

There are mainly three types of pathogens, infecting seed of different crops. These are as follows.

23.2.1 Internally Seed-Borne Pathogens

Pathogens establish relationship within the seed and inoculums present within the seed tissues that infect the seeds internally and destroy the endosperm and the embryo and affect seedling germination and development as in the case of loose smut of wheat and barley.

23.2.2 Externally Seed-Borne Pathogens

These pathogens are present on the seed surface superficially usually as spores, oospores, sclerotia, pieces of mycelium and chlamydospores as in the case of Karnal bunt of wheat, covered smut of barley, downy mildew of pearl millet, etc.

23.2.3 Admixture

Pathogens are independent of seeds but accompany them as concomitant contamination in the form of sclerotia, infected plant debris, nematode cysts, infected soil particles, etc., mixed with the seed. Ergot sclerotia get mixed with healthy seeds during threshing.

23.3 Chemical Seed Treatments

Some of traditional chemicals for the management of seed-borne diseases with traditional mode of action are sulphurs, coppers, benzimidazoles, strobilurins and sterol inhibitors.

23.3.1 History of Chemical Seed Treatment

In the seventeenth and eighteenth centuries, the brine solution was used as a seed treatment for the control of stinking smut.

In 1740, arsenic-based chemicals were used for seed treatment.

In 1755 and 1807, copper compounds were used for the management of stinking smut by Tillet and Prevost.

In 1808, arsenic compounds were banned for seed treatment.

In 1885, in India copper sulphate was first time used for the management of grain smut of sorghum.

In 1913, organo-mercurial fungicides were used for the management of stinking smut by Reihm.

In 1915, organo-mercurials were introduced for the chemical treatment of vegetable and small grain seeds.

In 1925, organo-mercurials were used as a seed treatment for the management of sorghum smut by Hilson.

In 1940 and 1943, chloranil and dichlone, two quinone compounds, were introduced as a seed protectant.

In 1941, thiram was introduced as a seed protectant.

In 1952, captan as a heterocyclic nitrogenous compound was introduced as a seed protectant.

In 1960, systemic fungicides like oxathin were introduced.

In 1966, carboxin was introduced for the management of loose smut of wheat.

In 1982, organo-mercurials were banned for seed treatment.

In 1990, modern fungicides were introduced for seed treatment.

Seed-borne pathogens are among the most important causes of disease problems, and, consequently, measures aimed at a reduction of these pathogens at the seed level are of key importance. The users tend to use higher than necessary doses of fungicides of their 'no risk' attitude towards chemical seed treatments. Restricting and better targeting of fungicide seed treatments will also contribute to the prevention of the currently widespread problems with fungicide resistance in cereal pathogens. The selection of resistant varieties is also a significant constituent in the preventive strategy. Loose smut of barley (*Ustilago nuda*) is mostly controlled by resistant varieties, and in Sweden, Stava is consequently grown as the predominant winter wheat variety. This variety is resistant to common bunt (*Tilletia tritici*) and dwarf bunt (*Tilletia controversa*), which are the most severe seed-borne threats to this crop (Borgen 2001). In barley, leaf stripe (*Pyrenophora graminea*) is the most severe disease. Efficient sources of resistance are present against this disease, but still there is short knowledge of which varieties are resistant and which are not. Thiram and seed disinfectants are seed treatments being used in the United States for the control of seed-borne pathogens. Thiram (tetramethylthiuram disulphide) is commonly used in nurseries as a bird and animal repellent, as well as a fungicide. In particular, thiram has been effective against *F. oxysporum* on ponderosa pine and Douglas-fir seeds (Littke 1997) and against *Fusarium* spp. on longleaf pine seeds (Barnett and Varela 2003). Some fungicides work most effectively if used before planting. Others may be applied after sowing or planting. Some of the narrow range chemicals are most effective in controlling a specific organism. Combinations are used to increase efficacy and host range (Vishnavat 2009). The development of effective seed treatments is one of the most significant advancements in plant disease management (Table 23.2).

Table 23.2 Some examples of chemicals used in the management of seed-borne diseases and their mode/scheduling of delivery

Crop	Disease	Chemicals	Reference	Schedule
Bean	White mould (*Sclerotinia sclerotiorum*)	Benomyl (Benlate) or thiophanate methyl (Topsin M)	Vieira et al. (2003)	The chemical must be applied when 80–100% of the plants have one or more flowers and small pods
	Ascochyta fabae	Azoxystrobin (0.1% a.i)	Nganga et al. (2016)	2 or 3 fungicidal sprays at flowering and seed setting to prevent infection
Rice	Blast (*Pyricularia oryzae*)	Difenoconazole (1.25 ml/l of H_2O)	Usman Ghazanfar et al. (2009)	At mid-tillering and boot stage
	Brown spot (*Drechslera oryzae*)	Propiconazole (0.1%)	Lore et al. (2007)	Spray at heading and grain maturation stage
Tomato and pepper	Bacterial blight of pepper and tomato (*X. campestris* pv. *vesicatoria*)	Copper hydroxide at 1.2 g/l + cymoxanil at 0.21 g /l + famoxadone at 0.46 g/l	Fayette et al. (2012)	Foliar spray first at the time of disease appearance and further spray at 15-day interval
Soybean	Purple seed stain (*Cercospora kikuchii*)	Carbendazim @ 0.2%	Chavan and Padule (2006)	Foliar sprays starting from flowering till full pod set at 7–10-day intervals

23.3.2 Classification of Fungicidal Seed Treatments

Fungicidal seed treatments can be classified based on association of the seed treatment product in relation to the seed. Fungicides used as protectants are effective against seed surface-borne pathogens and provide control against seed-borne pathogens. Various types of seed-borne pathogens, except those causing root rots, are controlled by protectant fungicides such as captan, maneb, thiram or fludioxonil (Table 23.3). Seedlings absorb systemic seed treatment fungicides as a result of which the growth of fungus inside the host plant tissues is inhibited. Systemic fungicides used for seed treatment include azoxystrobin, carboxin, mefenoxam, metalaxyl, thiabendazole, trifloxystrobin and various triazole fungicides, including difenoconazole, ipconazole and tebuconazole.

Water mould fungi *Pythium* and *Phytophthora* can be controlled by mefenoxam and metalaxyl. Biological agents as seed protectants are also available and may provide protection against these pathogens. Fungicides differ in their activity against different pathogens and only a few fungicides are available as seed treatments for every crop. For example, *Phytophthora* species and *Pythium* species are effectively controlled by fosetyl-Al (Aliette). It is absorbed by foliage and moves into roots. It

Table 23.3 Seed treatment chemicals for the management of seed-borne infection

Chemical	Active ingredient with dosage/Kg seed	Crop	Disease
Metalaxyl 35%WS	a.i (gm) 2.0–2.4 gm	Maize, bajra, sorghum, sunflower	Sorghum downy mildew, sugarcane downy mildew
Captan 50% WP	a.i 1–1.5 gm	Apple, cherry, grapes, coffee, tomato	Scab, brown rot, downy mildew, early blight, damping-off
Thiram 75%WS	a.i 2.5 gm	Groundnut, potato, rice, barley and cotton	Collar rot, Karnal bunt, loose smut
Carboxin 75% WP	a.i 1.5–1.875 gm	Wheat, barley, cotton	Bunt, loose smut, angular leaf spot

is used as a drench at 0.8–1.6 lb. a.i./1000 sq. ft. using 0.5–1.5 pt. water/sq. ft. The active component in SoilGard is *Gliocladium virens.* This fungus under certain conditions helps provide control of *Pythium* and *Rhizoctonia* fungi. Iprodione is used at seeding or transplanting as drench (1–2 pt./sq. ft.) at the rate of 0.2 lb. a.i./100 gal water. It is effective against *Rhizoctonia* damping-off, *Sclerotinia* and grey mould. Mefenoxam is active against only *Pythium*, *Phytophthora* and downy mildews. Mefenoxam granular formulation can be used before planting as soil application. It is water-soluble and readily leaches from soil. It is absorbed by plant parts including roots; movement in the plant is primarily by the xylem.

Diseases caused by *Rhizoctonia solani* and *Sclerotinia* spp. are effectively controlled by PCNB (now banned) also called quintozene and are the best available chemical for southern wilt caused by *Sclerotium rolfsii.* It is insoluble in water and must be thoroughly mixed with soil to reach its desired depth of control. It is inactive against *Pythium* pathogens. Some reports showed that germination of seeds may be inhibited and small plants may be stunted due to the use of this fungicide. Thiophanate-methyl is generally applied after sowing. It helps to control *Rhizoctonia* diseases, cottony rot, *Thielaviopsis* rots and some *Cylindrocladium* diseases. Thiophanate-methyl is absorbed by plant parts exposed to the chemical.

23.4 Proper Application and Use of Precautions

Fungicidal seed treatment products vary in formulation type, packaging and user's needs/desires. Products may be dry or liquid and in concentrate or ready-to-use formulations, while many seed treatments may be applied on-farm. There are several products that are to be used only by commercial applicators using closed application systems. The effectiveness of a fungicide to control disease depends on uniform fungicide coverage of the seed, and this is not easy to achieve in planter-applied situations. Always read and follow directions described on the label/leaflets provided. It is important to understand guidelines for proper application which are product-specific such as when and how to apply, feeding or grazing restrictions as well as important safety precautions. Always dispose of pesticide containers properly. Proper caution should be exercised when working with seed treatment pesticides. Handling precautions must be applied while handling seed treatment

fungicides as per the directions mentioned on label/leaflets about safe handling, mixing, storage and disposal because fungicides are highly poisonous and many are irritants. Personal protection, including standard chemical respirator, goggles and pesticide-resistant gloves, is recommended even if not specifically mentioned on the fungicide label. Application rates/doses as mentioned on the leaflets/labels must be adhered to because application of overdose may cause damage and under-application may reduce the efficacy of the fungicide. Properly calibrate all application equipment to assure uniform coverage. Uniform coverage of the seed ensures effectiveness of the seed treatment. A number of seed treatment methods are available, though not all are appropriate for every situation. Commercial application or application through dedicated seed treatment equipment will likely provide the most uniform coverage. Some of important chemical fungicides commonly used for seed as well as plant treatments are listed below (Table 23.4).

Table 23.4 List of important chemical fungicides commonly used for seed as well as plant treatments

Technical name	Chemical name	Trade name
Carbendazim	Methyl 1H-benzimidazol 2-yl carbamate	Bavistin 50% WP
Carbendazim + mancozeb	Sodium salt of aryl and naphthyl sulphonate	SAAF 12% + 63% WP
Hexaconazole	2-(2,4-Dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol	Contaf plus 5% SC
Captan	3aR,7aS-2-[(Trichloromethyl) sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione	Captaf 50% WP
Tebuconazole	(RS)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H,1,2,4-triazol-1-ylmethyl)pentan-3-ol	Raxil 2DS
Propiconazole	1-[[2-(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	Tilt 25 EC
Difenoconazole	1-[[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl] methyl]-1H-1,2,4-triazole	Score 25 EC
Vitavax	5,6-Dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide þ dimethylcarbamothioylsulfanyl N,N-dimethylcarbamodithioate	Trooper WS (carboxin 37.5% + thiram 37.5%)
Mancozeb	Zinc; manganese(2þ); N-[2-(sulfidocarbothioylamino)ethyl]carbamodithioate	Tata M-45 (mancozeb 75%)
Imazalil	(+)-Allyl-1-(2,4-dichlorophenyl)-2-imidazol-1-ylethyl ether	Double R, Deccozil
PCNB	Pentachloronitrobenzene	Terrachlor, Parflo, TerraFlo, Terrazan
Thiram	Tetramethylthiuram disulphide	Arasan, Vertagard
Maneb	Manganese ethylene-bis-dithiocarbamate	DB green, Granol NM, Trinox
Triadimenol	(1RS, 2RS; 1RS, 2SR)-1-(r-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl) butan-2-ol	Baytan
Triticonazole	(+)-(E)-5-(4-Chlorobenzylidene)-2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol	Charter

Table 23.5 Inherent risk of resistance associated with different fungicides and chemical classes of fungicides

Resistance risk	Chemical class or compound
High	Benzimidazoles, dicarboximides, phenylamides, strobilurin analogues (e.g. methoxyacrylates, oximino acetates)
Moderate	2-amino-pyrimidines, aminies (including morpholines), anilinopyrimidines, aromatic hydrocarbons, azoles, carboxanilides, carboxylic acid amides, carpropamid, cymoxanil, fenhexamid, kasugamycin, phenylpyrroles, phosphorothiolates, quinoxyfen
Low	Chlorothalonil, coppers, dithiocarbamates, fosetyl-Al, pyroquilon, phthalimides, sulphurs, tricyclazole

Source: Brent and Hollomon (2007a, b)

23.5 Fungicide Resistance Risk

The risk of resistance development mainly depends on the chemical class of the fungicide, the pathogen involved and how and where the treatment is made. Each chemical class is characterized by a typical resistance pattern (Brent and Hollomon 2007a, b). Table 23.5 categorizes the major chemical classes and compounds according to whether they are at high, moderate or low risk of resistance development (Brent and Hollomon 2007a, b).

23.6 Systemic Acquired Resistance Induced by Pathogen Infection

A second potential principle for chemically mediated disease control could be based on compounds that would induce the SAR response. The concept of a new principle for disease control provides an exciting prospect. The availability of an ongoing, broad-spectrum, stable solution to disease control would certainly have an enormous positive impact on food production. Furthermore, the SAR chemical should not exhibit direct activity against the microorganism. In the case of antimicrobial activity, an SAR-inducing activity could be established by demonstrating resistance to pathogen isolates that are genetically resistant to the antibiotic effects of the chemical. The first laboratory investigation of systemic acquired resistance was carried out by Ross in 1961. By inoculating *Tobacco mosaic virus* (TMV) on a local lesion host (Xanthinc tobacco), it was found that severity of successive infections was reduced. Ross coined the term SAR for the resistance that developed in the distal, unprocessed portions of plants inoculated with TMV. Broad-spectrum resistance to fungal, bacterial and viral pathogens has been found in tobacco due to SAR. It has not been found effective against aphids and tobacco hornworm (Walters and Fountaine 2009). In broad terms, induced resistance is of two types: systemic acquired resistance (SAR) and induced systemic resistance (ISR). Different agents,

viz. necrotizing pathogens and chemicals (e.g. acibenzolar-*S*-methyl, ASM), can be used to induce SAR which is mediated by a salicylic acid (SA)-dependent process (Spoel and Dong 2012). Plant roots are colonized by certain strains of plant growth-promoting rhizobacteria (PGPR) resulting in ISR which is mediated by a jasmonate (JA) and ethylene (ET) (Spoel and Dong 2012).

23.6.1 Salicylic Acid

Salicylic acid (SA) plays an important part in SAR-signal transduction after pathogen infection and is called as an exogenous inducer of PR protein accumulation and resistance (White 1979). SA is needed for SAR. But it is not the signal molecule that is translocated. These conjugates lack the phloem mobility of free salicylate. There are crop-tolerance issues with the use of SA. The safety margin between the rate at which the compound is effective and the rate at which it causes phytotoxicity is narrow (White 1979).

23.6.2 Agents That Induce Resistance

A wide range of biotic and abiotic agents induce resistance to pathogen infection (da Rocha and Hammerschmidt 2005; Lyon 2007). Probenazole was the first chemical resistance activator. It was registered as Oryzemate in Japan in 1975. Now numerous chemical and biological activators, viz. Milsana (*Reynoutria sachalinensis* extract; KHH BioScience); ASM, registered as Bion and Actigard (Syngenta); Messenger (harpin protein; Plant Health Care); and Elexa (chitosan; SafeScience), are available. In the sections below, agents demonstrated to have induced resistance to pathogens in various crops are listed (Table 23.6).

23.6.3 Chemical Activator

23.6.3.1 ASM

ASM has been found to induce SAR against a broad spectrum of pathogens in many species of plants. Induction of SAR in rust (*Uromyces viciae-fabae*) and *Ascochyta* blight (*Ascochyta fabae*) of faba bean under glasshouse and field conditions has been reported by application of ASM (Sillero et al. 2012). Due to SAR, activities of defence-related enzymes and phenolic compound increased. Priming of defence enzymes was observed when both susceptible and resistant genotypes were treated with ASM (Barilli et al. 2010). Expression of gene and peroxidase and polyphenol oxidase activities were increased, but ASM effectiveness was determined by variety (Lin et al. 2011). However, in groundnut, increased infection of *Cercosporidium personatum* was found due to ASM (Zhang et al. 2001).

Table 23.6 Some examples of host resistance inducer and target pathogens

Resistance inducer	Protected plant	Targeted pathogen	Reference
Acibenzolar-*S*-methyl (ASM)	Cucumber	*Colletotrichum lagenarium*	Du et al. (2012)
	Cabbage	*Peronospora parasitica*	van der Wolf et al. (2012)
	Tomato	*Ralstonia solanacearum*	Hong et al. (2011)
	Turmeric	*Pythium aphanidermatum*	Radhakrishnan et al. (2011)
	Rice	*Xanthomonas oryzae*	Du et al. (2012)
	Maize	*Bipolaris maydis*	Du et al. (2012)
	Pea	*Uromyces pisi*	Barilli et al. (2010)
β-Aminobutyric acid (BABA)	Apple (post-harvest)	*Penicillium expansum*	Quaglia et al. (2011)
	Pea	*Uromyces pisi*	Barilli et al. (2010)
	Tomato	*Phytophthora infestans*	Sharma et al. (2012)
Probenazole	Maize	*Bipolaris maydis*	Yang et al. (2011)
Saccharin	Soybean	*Phakopsora pachyrhizi*	Srivastava et al. (2011)
Potassium phosphite	Grapevine	*Plasmopara viticola*	Pinto et al. (2012)
	Arabidopsis	*Phytophthora cinnamomi*	Eshraghi et al. (2011)
Silicon	Rose	*Podosphaera pannosa*	Shetty et al. (2012)
Biochar	Pepper	*Leveillula taurica*	Elad et al. (2010)
	Asparagus	*Fusarium oxysporum*	Elmer and Pignatello (2011)

23.6.3.2 β-Aminobutyric Acid

A non-protein amino acid, β-aminobutyric acid (BABA), has been reported to induce resistance against an array of pathogens on different crop plants. Cohen et al. (2010) showed that BABA-induced resistance is not due to the role played by SA, JA and abscisic acid. Application of BABA, one day after inoculation caused induced hypersensitive effect in penetrated epidermal cells, whereas its application 2 days after inoculation resulted in encasement of the primary haustoria of the pathogen with callose. Late blight pathogen *Phytophthora infestans* could be controlled by BABA (Sharma et al. 2012), and the *Plasmopara viticola* severity on grapevine was reduced by 62% in field experiments (Tamm et al. 2011). BABA-induced SAR in *Brassica napus* against *Leptosphaeria maculans* pathogen is also well documented. It also induced synthesis of SA and PR-1 expression and was also found to exert a direct fungitoxic effect against the pathogen (Šašek et al. 2012).

23.6.3.3 Probenazole

For more than three decades, probenazole has been used to manage rice blast (*Magnaporthe grisea*) and bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) pathogens (Iwata 2001). Despite its widespread use, there has been no problem of development of resistance in the target pathogens (Iwata 2001). In *Arabidopsis*, it has been reported that a site upstream of the point of accumulation of SA in the

SAR-signalling pathway is stimulated by its active metabolite 1,2-benzisothiazole-1,1-dioxide to induce SAR (Yoshioka et al. 2001). Control of southern maize leaf blight due to *Cochliobolus heterostrophus* in maize field and glasshouse without adversely affecting plant growth has been recently demonstrated (Yang et al. 2011).

23.6.3.4 Saccharin

It is a metabolite of probenazole (Uchiyama et al. 1973). It has been found to induce SAR against *M. grisea* and *X. oryzae* which are important pathogens of rice (Siegrist et al. 1997; Oostendorp et al. 2001) and also against broad bean rust (Boyle and Walters 2005) and powdery mildew and *Rhynchosporium commune* on barley (Boyle and Walters 2006; Walters and Fountaine 2009).

23.6.3.5 Phosphite

For more than 30 years *Phytophthora* diseases in different species of plants have been managed by using phosphite (Hardy et al. 2001). However, the mode of action of phosphite is complex, and it inhibits growth of pathogen and stimulates host defences directly (Daniel and Guest 2006). The recruitment of a broad array of host defences was found based on activation of defence genes from both the SA and JA/ET pathways.

23.6.3.6 Biochar

Biochar is a product of pyrolysis (Laird 2008). There is a report on improvement of crop performance due to increasing nutrient preservation, when soil is modified with biochar (Chan et al. 2007), enhancing mycorrhizal fungi in soil (Warnock et al. 2007) and changing populations and functions of soil microbes (Steiner et al. 2008). Its application in soil has been shown to induce systemic resistance to grey mould (*Botrytis cinerea*) on pepper, powdery mildew (*Leveillula taurica*) on tomato and the broad mite pest (*Polyphagotarsonemus latus*) on pepper (Elad et al. 2010).

23.6.4 Other Potential Resistance-Activating Chemicals

Certain chemicals or extracts, viz. fosetyl-AI, metalaxyl (Ward 1984) and triazoles (Hauthal 1993), have been reported to exhibit resistance-inducing molecules, but it has not been demonstrated as these compounds meet the criteria for SAR activators. Fosetyl-AI shows very weak antifungal activity in traditional in vitro assays (Bompeix et al. 1980). Plants pretreated with this compound accumulate phytoalexins faster (Guest 1984) and the effectiveness of metabolic inhibitors resembling glyphosate. Based on these studies, it was assumed that fosetyl-AI induces resistance. Strong in vitro activity is indicated when phosphate is not present in synthetic media. This is probably due to the interaction of phosphoric acid with intracellular phosphate metabolism. Fungal strains selected for insensitivity to fosetyl-AI in vitro were not controlled by fosetyl-Al in planta. This was a proof against the ability of fosetyl-AI to induce resistance (Fenn and Coffey 1985; Dolan and Coffey 1988). 2,2-Dichloro-3,3-dimethylcyclopropane carboxylic acid (DCP) has been reported as a compound for the control of rice blast which was described by Langcake and

Wickins (1975a, b) as a compound for specific rice blast control. It is mildly antifungal in vitro and is able to induce some peroxidase activity in treated plants. Upon infection in rice, plants treated with DCP accumulated momilactone phytoalexins faster and to higher levels than untreated controls as observed for all agents that inhibit melanin biosynthesis since melanin is important. This compound showed specificity for melanin-forming fungi along with the in vitro effects on melanization, the absence of positive correlation with a biological model of SAR and the failure to induce chitinase in cucumber signal that DCP may inhibit melanin biosynthesis (Langcake and Wickins 1975a, b).

Probenazole has been used to protect rice against *Pyricularia oryzae* and *Xanthomonas oryzae*. It exhibits only very weak in vitro activity. These factors were identified as fungitoxic, unsaturated fatty acids, including linolenic acid. After blast fungus infection, a number of enzymes possibly playing part in defence of plant showed much higher activity in probenazole-treated plants than in untreated, infected controls (Iwata et al. 1980). Probenazole also potentiated the 'respiratory burst' and accumulation of superoxide anion radical normally observed after infection with blast fungus (Sekizawa et al. 1985). However, lipoxygenase which is a molecular marker for SAR in rice is not induced significantly by probenazole (C. Hofmann, unpublished data). Therefore, the correlation between probenazole and its biological marker for SAR has not been established, despite its effectiveness against *P. oryzae*. In laboratories, 2,6-dichloro-isonicotinic acid and its methyl ester have also been reported and are able to induce systemic resistance in plants (Kessmann et al. 1993).

23.7 Future Prospects

Disease management still depends on chemical control, but the frequent occurrence of fungicide resistance may increase in the future because the choice of fungicides and its uses are often difficult when effective alternatives are scanty. Development and integration of disease management tools along with safe chemicals need to be accelerated to alleviate public concerns about agricultural chemicals. A number of alternatives have been developed to reduce the use of pesticides, often driven by environmental reasons. Fungicide treatment of seeds is an area where possibilities of environmental pollution are always associated and it also affects the non-target and beneficial organisms in the nature. Hence, there is a need to develop seed treatment chemicals that are eco-friendly, target specific and have a wide spectrum of activities against seed-/soil-borne pathogens.

23.8 Conclusion

Different chemicals are available to control plant diseases by inhibiting the growth of or by killing the disease-causing pathogens. Bactericides, fungicides and nematicides may be applied to seeds to prevent or reduce infections by utilizing various

principles of disease control. Eradicants are designed to kill a pathogen that may be present in/on the seeds, or on vegetative propagative organs, such as bulbs, corms and tubers. Protectants place a chemical barrier between the pathogen and plant. Therapeutic chemicals check an infection in progress. A re-registration process requires that agriculturally important chemicals fulfil the demand of concerned regulatory authorities regarding low toxicity to humans, cattle and wildlife, low environmental impact, low residues in food and so on. The public and farmers also demand compatibility with IPM programmes. The agricultural chemical companies take these demands into account for deciding which fungicide to develop and commercialize, and the likelihood of discovery becomes lower. This change will then limit farmers' choices for products. By contrast, the fight against pathogens that adversely affect food production will continue in the future. It is important to have fungicides with diverse modes of action against seed−/soil-borne pathogens and develop suitable seed treatment or seed pelleting techniques based on present cumulative knowledge.

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Biotechnology: An Intervention for Genetic Resistance Against Seed-Borne Phytopathogens

24

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Abstract

Agriculture contributes significantly to the world economy, as 70% of the population is engaged in agriculture and allied activities. Wide array of plant diseases highly impact crop production and its quality. Losses triggered by diseases vary from slight to severe depending on crop and existing environmental conditions. Plant pathogens are seed transmitted and seed is an efficient means of introducing plant pathogens into a new area, thus providing a means of their survival from one cropping season to another. In present global scenario, seed-borne diseases are of great importance because seed has become an international commodity and contribute more than ever for the movement of plant pathogens across capacious distances, natural barriers and international border. Each year, seeds are exchanged throughout the world by commercial seed trading activities, through germplasm exchange activities and by public and private institutes concerned with crop improvement. Presently, seed-borne phytopathogens pose a serious threat to agriculture production and their close association with seeds, facilitating their long-term survival, introduction into new areas and their widespread distribution. Seed-borne pathogens and diseases continue to be problematic and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas which cause significant economic losses worldwide. So, there is a huge need for improving the strategies for management of these diseases. In recent years, biotechnology, as a science, has seen many advances and high-throughput tools and techniques, which are being used in many spheres of crop improvement. Various biotechnological tools ranging from marker-aided selection, plant tissue culture and genetic engineering techniques have been successfully used for improvement of many crop plants.

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Similarly approaches for detection of seed-borne diseases and their management have been playing a key role in the massive production of improved and disease-resistant crop varieties, as well as in their genetic improvement.

24.1 Introduction

Food security along with sustainability in the twenty-first century is the greatest defiance for the researchers. One of the alarming challenges in front of agriculture is to double the food production to feed an ever-increasing human population which is estimated to increase from the present 1.15 billion to 1.6 billion in India and from 6.8 to 9.2 billion globally by 2050 (FAO 2011). During the World Food Summit in Rome in 1996, all participant countries agreed to reduce the number of hungry people by 2015 but still there are 800 million starving people, almost all of them in developing countries. It is not only important to produce more but also to protect what we produce. It has long been recognized that plant diseases are one of the important constraints to crop yields, due to which crops do not reach its potential which is determined by genetic and environmental limitations (Gaunt 1982). At a conservative estimate, 35 to 40% of our crop yields valued about ₹600 billion are lost due to pests, diseases and weeds annually despite using chemicals for their control costing around billions each year (Manjunath 2010).

Among the major factors which influence agricultural productivity, seed has a place of prime importance, since seed is the carrier of the genetic potential for higher crop production. Plant diseases have been found to affect the growth and productivity of crop plants because many pathogens can survive in, on or with seeds. A vast number of plant pathogens from viroids of a few hundred nucleotides to eukaryotic fungi cause seed-borne or transmitted diseases in crops (Table 24.1). Thus, seeds play a vital role for the healthy production of a crop. These pathogens cause diseases at various stages of crop growth from germination of seed up to crop maturity. In wheat alone they are responsible for reducing yield up to 15–90%, if infected and untreated seeds are grown in the field (Wiese 1984). Similarly, rice is known to be affected by as many as 36 seed-borne pathogens (Ou 1985). Seed-borne pathogens cause different types of diseases, e.g. seed and seedling rots, root rot, stem rot, fruit rot, wilt, blight, leaf spot, etc., leading to pre- and post-emergence losses and at various stages of crop growth.

Seed-borne diseases may affect crop productivity in two ways. Firstly, seeds containing viable inoculums may introduce new pathogenic species or races to areas at local, regional, national or international levels and, secondly, an economic loss which exacerbates the current deficit of food supply. Economic costs of a seed-borne disease include the direct (yield and quality) costs and costs of the control measures and impacts of trading restrictions such as quarantines or embargoes imposed by purchasers and also cause problems for the seed production and distribution industry.

Karnal bunt is a fungal disease of wheat which is caused by *Tilletia indica*. It causes significant economic losses in wheat crops due to quarantine and other

Table 24.1 Diseases of economic importance, their causal organism and relevant hosts

Crops	Diseases	Pathogen
Wheat	Loose smut Karnal smut Flag smut	*Ustilago segetum* var. *tritici* *Neovossia indica* *Urocystis agropyri*
Chickpea	Ascochyta blight Wilt	*Ascochyta rabiei* *Fusarium oxysporum* f.sp. *ciceri*
Crucifers	Grey and black leaf Spot	*Alternaria brassicae* *Alternaria brassicicola*
Rice	Bunt False smut Blast	*Neovossia horrida* *Ustilaginoidea virens* *Pyricularia oryzae*
Cotton	Anthracnose Wilt	*Colletotrichum indicum* *F. oxysporum* f.sp. *vasinfectum*
Maize	Black kernel rot Cob rot Southern leaf blight	*Botryodiplodia theobromae* *Fusarium moniliforme* *Drechslera maydis*
Pearl millet	Downy mildew Smut	*Sclerospora graminicola* *Tolyposporium penicillariae*
Sorghum	Anthracnose Kernel or grain smut Downy mildew	*Colletotrichum graminicola* *Sphacelotheca sorghi* *Peronosclerospora sorghi*
Soybean	Anthracnose Pod and stem blight Purple seed stain	*Colletotrichum dematium* *Phomopsis sojae* *Cercospora kikuchii*
Cucumis spp.	Anthracnose	*Colletotrichum lagenarium*
Brinjal	Fruit rot	*Phomopsis vexans*
Carrot	Black root rot	*Alternaria radicina*
Onion	Damping off Downy mildew Purple blotch Stemphylium blight	*Botrytis allii* *Peronospora destructor* *Alternaria porri* *Stemphylium vesicarium*
Pepper chilies	Anthracnose	*Colletotrichum capsici*
Radish	Grey leaf spot Leaf spot	*Alternaria brassicae* *Alternaria raphani*
Tomato	Buck eye rot Damping off Early blight Late blight	*Phytophthora parasitica* *Pythium aphanidermatum* *Alternaria solani* *Phytophthora infestans*

export restrictions placed on infected areas. Some species like common wheat, durum wheat and triticale are mainly affected by this disease (Royer et al. 1986; Warham et al. 1986). The estimation of loss by Karnal bunt is difficult because it affects grain quality. Rigorously infected grains produce weak seedlings that will show considerable reduction in the viability of seeds (Rai and Singh 1978).

Loose smut is also a seed-borne disease of wheat and barley and the causal organisms of the disease are *Ustilago segetum* var. *tritici* and *U. nuda*, respectively. It is a serious problem of humid and semi-humid areas. The disease results in the

reduction of yield from 20% to 50. It is a threat to seed production in developing countries where small-scale farmers use their own harvest as seed material. Elimination of this disease can be done by the use of foliar fungicides that eliminate the fungus from infected plants (von Schmeling and Kulka 1966; Jones 1999).

Common root rot is another serious problem in wheat; infected seeds play an important role in the spread of this disease. *Cochliobolus sativus*, *Fusarium culmorum* and *F. graminearum* are the causal pathogen in wheat (Harding 1971, 1972, 1979). Decreased number of tillers formed on an infected plant is the indirect symptom of common root rot.

One of the major yield-reducing factors in crop plants is plant viruses, consisting of DNA or RNA. There are 92 genera of plant viruses of which 82 were assigned in 21 different families as reported in International Committee on Taxonomy of Viruses (King et al. 2012). Seed-transmitted viruses include *Alfamovirus*, *Necrovirus*, *Tombusvirus*, *Bromovirus*, *Capillovirus*, *Potyvirus*, *Sobemovirus*, *Carlavirus*, *Tobamovirus*, *Caulimovirus*, *Ilarvirus*, *Tospovirus*, *Carmovirus*, *Comovirus*, *Cryptovirus*, *Cucumovirus*, *Enamovirus*, *Fabavirus*, *Furovirus*, *Hordeivirus*, *Nepovirus*, *Potexvirus*, *Tobravirus* and *Tymovirus* (Sastry 2013). The *Caulimovirus* is a double-stranded DNA virus while geminivirus is a single-stranded DNA virus, *Reoviridae* is a double-stranded RNA virus and potyvirus is a single-stranded RNA virus. Genome of plant virus groups commonly consists of RNA (Brunt et al. 1996). Half of the reported emerging infectious diseases from plants are caused by viruses (Anderson et al. 2004). The yield losses that can be ascribed to plant viruses are estimated to cost worldwide more than $30 billion yearly (Sastry and Zitter 2014). In South East Asia more than $1.5 billion yield losses are estimated due to virus-affected rice culture (Abo and Sy 1997; Hull 2013; Sasaya et al. 2013). The extent of losses due to major seed-borne diseases in crop plants can be accessed from Table 24.2. Pathogen detection and disease diagnostics, a key factor of any crop management programme, is also useful in observing sanitary and phytosanitary measures to seed quality. Molecular diagnostics based on immunological and DNA techniques can provide an effective approach for disease surveillance and predicting disease occurrence and intensity.

Despite substantial advances in plant disease management strategies, global food supply is still threatened by a dynamic nature of pathogens and pests. This changed scenario warrants us to respond more efficiently and effectively to this issue. This situation demands judicious blending of conventional, unconventional and frontier technologies (Fagwalawa et al. 2013) for the management of diseases. Biotechnology offers exciting opportunities to overcome biotic and abiotic stresses in crop plants and support to enhance agricultural productivity on a sustainable basis. Biotechnology offers the genetic manipulation and multiplication of any living organism through novel techniques and tools such as tissue culture and genetic engineering in order to produce new organisms and/or products that can be used in variety of ways.

It is assumed that disease resistance is the most effective means of controlling disease. However, there are many pathogens for which no effective sources of disease resistance have been identified. In this context, biotechnological approaches can be used to determine the type and sources of host resistance. Many countries,

Table 24.2 Worldwide impact of some seed-borne disease in crop plants

Crop	Disease	Country	Economic loss	References
Wheat	Karnal bunt	India	~20 to 40% yield loss	Vocke et al. (2002)
		Northwestern Mexico	~39% yield loss	Ahmed et al. (2013)
	Fusarium head blight	North Dakota and Minnesota	~55% yield loss	Nganje et al. (2007)
		Kansas State	~61% yield loss	Matny (2015)
		North America	~19–22% yield loss	Salgado (2014)
		Argentina	~70% yield loss	Carranza et al. (2008)
Rice	Blast	USA	~30% rice production losses globally	Nalley et al. (2016)
		Greece	Up to 11% loss of yield	Koutroubas et al. (2009)
		Asia, W. Africa	~80% yield loss	Mew et al. (1993)
		Philippines	~50–70% yield loss	Disthaporn (1994)
Sorghum	Smut of sorghum	Madhya Pradesh and Gujarat	~25% yield reduction	Hafiz (1958)
Phaseolus vulgaris	*Bean common mosaic virus*	USA and Canada	~83% yield reduction	Sastry and Zitter (2014)
Pea	*Pea seed-borne mosaic virus*	Poland, Switzerland, New Zealand, UK, India, Morocco and France	~10–35% yield reduction	Maury and Khetrapal (1992)
Urdbean	*Urdbean leaf crinkle virus*	Pakistan	~81% yield reduction	Bashir et al. (1991)
Soybean	*Soybean mosaic virus*	Pakistan	~93% yield reduction	Ali and Hassan (1992)
	Tobacco bud blight	India	~66.43% yield loss	Dhingra and Chenulu (1980)
Tomato	*Tomato mosaic virus*	Iran	~94% of the seed loss	Broadbent (1976)
	Tomato spotted wilt	Tropical and subtropical	~64% yield reduction	Sharma (1999)
Chickpea	*Cucumber mosaic virus*	New Zealand	~80% yield loss	Jones et al. (2008)

including India, are engaged in research on about 55 crop species to incorporate transgenes to endow various traits such as resistance to pests, diseases or herbicides; tolerance to environmental stresses in crop plants; etc. (Manjunath 2010).

It is mandatory for the exporting countries to meet the challenge posed by Karnal bunt. In the USA, there is serious concern about the probability of export losses due to the recent introduction of Karnal bunt in Arizona, Texas and New Mexico

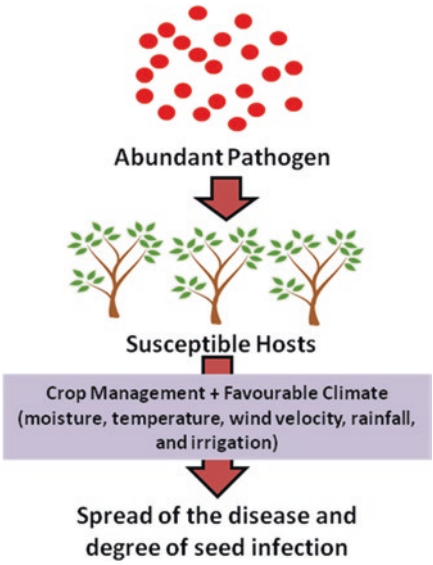

Fig. 24.1 Factors for spread of disease in an area

(Ykema et al. 1996). *T. indica* has therefore been declared as a quarantine pest by the USA. The European and Mediterranean Plant Protection Organization (EPPO/EOEPP) has therefore executed many standards for regulating diseases in the international trade. EPPO in 1996 prepared a list of 46 A1 quarantine organisms including *T. indica* (Kehlenbeck et al. 1997). When the USA strengthened its quarantine regulations in 1997 against KB, Mexico forced domestic quarantine regulations and also lawful restrictions on planting wheat in areas with high incidence of KB. Despite all these efforts an effective disease management strategy demands stepping up the disease surveillance programme by using modern molecular tools. Mode and factors of spread of a disease in an area have been explained (Fig. 24.1). Even if the transmission rates are low, pathogen load on, in and with seeds is an important determinant for disease initiation.

24.2 The Genetics of Resistance in Seed-Borne Phytopathogens

The genetic characterization of disease resistance in plants has been essential for the understanding of host-pathogen interactions. As we know, resistance and susceptibility are not absolute attributes of any host. Thus, understanding the genetic underpinnings of disease resistance in plants will thereby encourage a number of new and exciting opportunities for engineering resistance. Biffen (1905) studied the inheritance of resistance of a plant to its pathogen and suggested that Mendel's laws of inheritance were also applicable to resistance of plants to their pathogens.

The complementary nature of the genetics of host and pathogen in plant diseases was established by Flor in 1956, working on flax and its rust fungus *Melampsora*

lini, and formulated the gene-for-gene concept. The simplest statement of this fundamental concept is that "for every allele specifying resistance in the host there is a corresponding allele specifying avirulence in the pathogen". More often, resistance is inherited as a dominant rather than as a recessive character. But exception always exists, in particular, genes at the *Mlo* locus of barley which confer resistance to mildew (*Erysiphe graminis*) are recessive.

Molecular genetics of disease resistance in plants is one of the most challenging aspects of plant pathology. Molecular corroboration of the gene-for-gene concept has been validated by conversion of compatibility to incompatibility by transformation of susceptible plants with resistance genes and virulent pathogens with avirulence genes. However, this concept provided an excellent model for understanding specific resistance. In its simplest form, dominant avirulence alleles are recognized by cognate dominant resistance alleles in the plant but by no means all plant-pathogen systems should fit this simple model. Because populations of a plant species may vary quantitatively in their resistance to a given pathogen, such variation is often thought to be polygenic, each locus making a limited contribution to resistance. This raises the possibility that the appropriate combination of these genes would give a high level of resistance, which, owing to its multigenic nature, would be durable. One way of studying such resistance is by the use of quantitative trait loci (QTL) mapping (Young 1996).

So, two different genetic mechanisms for disease resistance have long been recognized: (i) complete resistance conditioned by a single gene (monogenic resistance) and (ii) incomplete or quantitative resistance conditioned by multiple genes having partial resistance. These resistance types in their extreme forms are clear and easily distinguished. A variety of terms have been used to refer to this perceived dichotomy, including horizontal versus vertical, complete versus incomplete, major gene vs minor gene, qualitative vs quantitative resistance, etc.

24.2.1 Breeding for Resistance

New methodologies in plant science offer tremendous potential for management of crop diseases. Among the various strategies, disease resistance is of immense practical importance. Use of cultivars with acceptable level of resistance to major diseases is considered as economically safe and best approach, as it can reduce or eliminate the expense and the negative effects of other chemical, physical, biological, cultural and regulatory control methods.

The most important objective to control the seed-borne disease is to limit the amount of primary inoculum. Thus, breeding disease-resistant varieties is a very successful and effective measure against these diseases. In essence, resistance is able to replace the chemical seed treatment but only minor efforts have been made regarding seed-borne diseases and knowledge in this area is limited. Benefits of using the plant resistance genes in breeding programs involve efficient reduction of pathogen growth, minimal damage to the host plant, zero input of pesticides from the farmers and most importantly the environment-friendly approaches. Traditional

approaches have several limitations; the introgression of resistance genes from one species into the gene pool of another by repeated backcrossing is a long-term process which usually takes many generations for introduction. In order to develop cultivars with built-in resistance, the knowledge about the methods of assessing the levels of resistance to diseases caused by seed-borne phytopathogens and identifying resistance sources or genes is essentially required.

24.3 Application of Biotechnology in Resistance Strategies

The genetic basis of resistance to various diseases affecting crops, nature of plant-pathogen interaction and markers for identifying genotypes possessing resistance genes can be studied easily nowadays by applying an array of molecular techniques (Collard and Mackill 2008). In this regard, advances in molecular biology, structural and functional genomics, bioinformatics and related fields have led to the development of driven tools, methods and products that promise to increase the speed of plant breeding research towards disease resistance. Biotechnological methods such as genetic recombination and marker-assisted selection (MAS) and tissue culture confer a number of advantages over conventional breeding techniques.

24.3.1 Marker-Assisted Selection

The fundamental basis of plant breeding is the selection of specific plants with desirable traits. Effective selection for disease resistance requires a large number of genes segregating in a population. In a bigger population size, accurate and cost-effective screening methods permit rapid testing of thousands of plants in order to identify specific gene combinations. Breeders have successfully developed cultivars resistant to diseases by integrating *R* genes into their cultivars for many years; but a durable, long-lasting resistance in many cases has been difficult to achieve as pathogens quickly evolve and develop counter-resistance genes that abrade the host cultivar resistance. Breeders often spot this breakdown in resistance and hurriedly integrate a newly found effective *R* gene into their populations.

In this regard, MAS offered advantage over other selection systems since it allows significant saving of space, time and money with more accuracy. Recently plant breeding relies on molecular markers as explained in Fig. 24.2. A marker is a "genetic tag" that identifies a particular location within a plant's DNA sequences. Markers can be used in transferring a single gene into a new cultivar or in testing plants for the inheritance of many genes at once.

Tanksley et al. (1981) have first demonstrated the real MAS-based selection on metric characters using isozyme markers in early generations in tomato lines. The advent of molecular markers provides a dramatic improvement in the efficiency and precision with which breeders can select plants with desirable combinations of genes. The recent developments in DNA marker technology have helped to develop

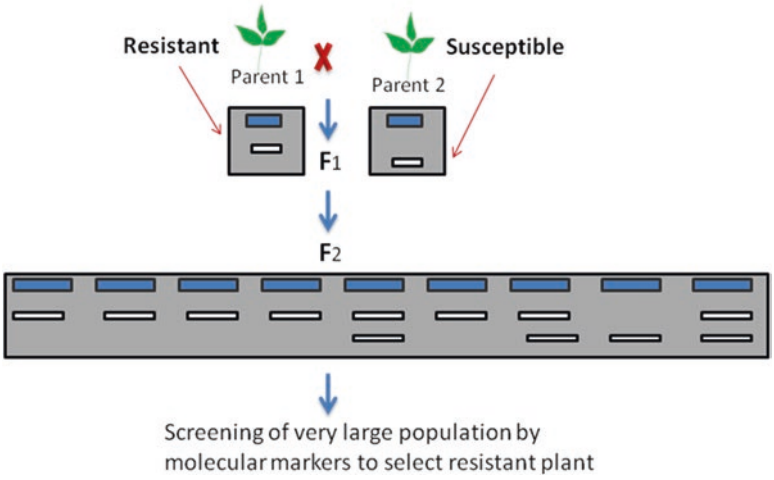

Fig. 24.2 Schematic representation of marker-assisted selection for resistance

the concepts of QTL mapping, MAS and genetic transformation to produce plants of desirable resistance.

For example, in the last few years high-density molecular linkage maps of only rice containing approximately 3000 markers have been developed and making the marker density in the rice genome, on average, one marker per centimorgan (Causse et al. 1994; Harushima et al. 1998; Lopez-Gerena 2006). For instance, many MAS rice genotypes for bacterial blight in Asia have been commercially released, e.g. Angke (Indonesia, 2002), Tubigan 11 (Philippines, 2006), Improved Pusa Basmati-1, Samba Mahsuri and Mahsuri Hybrids (India, 2007, 2011, 2013 respectively). Marker-assisted breeding for seed-borne diseases in cereals is listed in Table 24.3.

24.3.2 Use of Tissue Culture

Almost all tissue culture techniques are used in plant pathology. Problems are generally encountered if an effort is made in crossing distantly related species. Culturing of embryos developed from wild crosses before they get aborted is generally done by tissue culture using suitable growth medium.

Tissue culture of disease-resistant plants is particularly useful with clonally propagated plants such as strawberries, apples, bananas, sugarcane, cassava and potatoes. Prolific plantlet production from meristem and other tissue cultures facilitates the rapid propagation of plants with resistant genotypes, especially in those crops not propagated easily by seeds. An even greater use of tissue culture is for the production of pathogen-free stocks of clonally propagated susceptible plants (Agrios 2005). There are more than 200 plant virus and viroid diseases which are reported to be seed transmitted from different parts of the world and known to be the most efficient vehicles of transport for a number of phytopathogens. For

Table 24.3 Marker-assisted breeding in cereals for seed-borne diseases

	Disease name	QTLs	Markers	References
Wheat	Fusarium head blight	Qfhs.ndsu-3*BS* and Qfhs.ndsu-3*AS*, *Fhb1*, *Qfhs.ifa-5A*, *Fhb2*	SSR	Chen et al. (2000), Anderson et al. (2001), Liu et al. (2005), Anderson et al. (2007), and Salameh et al. (2011)
	Karnal bunt	QTLs, Qkb.dwr-5BL.1	SSR	Brooks et al. (2006), Kumar et al. (2007), and Kaur et al. (2016)
	Loose smut	*Ut6*, Utd1	SSR	Randhawa et al. (2009) and Kassa et al. (2014)
	Flag smut	*Fs.sun-3AL*, *QFs.sun-6AS*, *QFs.sun-1BL* and *QFs.sun-5BS*	SCAR	Toor et al. (2013)
	Cereal cyst nematode	*Cre3*, *CreX* and *CreY*	RAPD, SCAR	Martin et al. (2004) and Barloy et al. (2007)
Barley	*Barley yellow mosaic virus*	rym4, rym9, rym11	RAPD, SSR	Werner et al. (2005)
	Barley yellow dwarf virus	Yd2	STS	Jefferies et al. (2003)
	Loose smut	*Un8*	SSR, STS	Li et al. (2000) and Eckstein et al. (2002)
	Covered smut	*Ruh*	RAPD, SCAR	Ardiel et al. (2002)
Rice	Bacterial blight	Xa5, Xa13, Xa4 and Xa21	STS, CAPS, SSR, RFLP and AFLP	Huang et al. (1997), Chen et al. (2000), Sanchez et al. (2000), Chen et al. (2001), Singh et al. (2001), and Joseph et al. (2004)
	Blast disease	Pi1, Piz-5, Pita, pi66(t), Pi-z, Pi-9(t), OsGLP2–1, OsALD1	RFLP, STS, ISSR, SSR	Hittalmani et al. (2000), Jung et al. (2016), Liang et al. (2016), and Liu et al. (2016)
	Brown spot	qBSfR1, qBSfR4 and qBSfR11, qBS9, BSq2.2v and I, BSq2.1v and I, BSq6.1v, BSq6.2i, BSq8.1i, BSq8.2v, BSq11.1v and I, bs1	QTL	Sato et al. (2008a, b, 2015), Banu et al. (2008), and Katara et al. (2010)

(continued)

Table 24.3 (continued)

	Disease name	QTLs	Markers	References
	Bacterial seedling and grain rot	RBG1, (qRBS1), RBG2, qBPB-1-1, qBPB-1-3, qBPB-3-1 and qBPB-3-2, qBPB-2-1, qBPB-2-2	QTL	Pinson et al. (2010) and Mizobuchi et al. (2013)
Maize	Downy mildew	QTL	SSR, Gene Pyramiding	George et al. (2003)
Gene pyramiding				
Rice	Blast and bacterial blight	(Xa21 and Xa33 + Pi2 and Pi54), (Xa21+ Piz), (*Xa7*+ *Xa21* + *Xa22* + *Xa23*)	SSR	Narayanan et al. (2002) and Abhilash Kumar et al. (2016)
	Blast	*Pid1*, *Pib*, *Pita* and *Pi2*		Chen et al. (2004)
Wheat	Blotch and head blight	broad-spectrum resistance (BSR) QTL	SSR	Miedaner et al. (2012)

instance, meristem shoot and tip culture are very successful in eliminating the virus from infected germplasm and this technique has been remarkably exploited in banana, potato, cassava, sweet potato and many ornamental plants for the production of virus free plants. Other plant tissue culture techniques, e.g. protoplast fusion, are also employed for the development of disease-resistant plants. Maloy (2005) reported the successful development of disease-resistant plants by this technique in *Lactuca sativa* for downy mildew (*Bremia lactucae*), *Brassica napus* and *B. nigra* for blackleg (*Phoma lingam*) and *S. brevidens* and *Solanum tuberosum* against bacterial soft rot (*Erwinia* spp).

24.3.3 Transgenic Approach

By using transgenic approach, resistance genes across the species can be pyramided for the development of resistant crops. Traditional breeding for resistance against seed-borne disease was not applicable as crossability barriers were a big problem. The primary benefit of deploying resistance genes in transgenic technology is its ability to overcome the fertility constraints for the dispersal of genes originating from a different species; for example, Bs2 resistance gene was identified originally in pepper and its resistance has been found durable in the field against isolates of *Xanthomonas campestris* pv. *vesicatoria* that contain the corresponding bacterial avirulence gene avrBs2 and a functional expression of *Bs2* in stable transgenic tomatoes supports its use as a source of resistance in other solanaceous plant species (Tai et al. 1999). Resistance through transgenic approach can be derived by applying the following two approaches.

24.3.3.1 Pathogen-Derived Resistance (PDR)

The development of new recombinant DNA technology and efficient transformation techniques have allowed to design and test a number of biotechnological strategies

Table 24.4 Transgenic plant developed with pathogen-derived resistance for seed-borne diseases

Seed-borne disease	Transgene used
Papaya ringspot virus, *squash mosaic virus*, *alfalfa mosaic virus*, *beet necrotic yellow vein virus*, *potato leaf roll virus*, *potato virus Y (PVY). PVX*, *plum pox virus*, *rice stripe virus*, *citrus tristeza virus*, *soybean mosaic virus*, *common bean mosaic virus*, *pigeonpea sterility mosaic virus*	Coat protein
Banana bract mosaic virus, *bean golden mosaic virus*, *barley yellow dwarf virus*, *mungbean yellow mosaic virus*, *tobacco etch virus*, *potato spindle tuber viroid*, *bean pod mottle virus*, *pea early browning virus*, *tomato golden mosaic virus*	RNAi
Potato leaf roll virus, *PVY*, *PVX*, *pea seed-borne mosaic virus*	Replicase
Rice dwarf virus	Ribozyme
Tomato bushy stunt virus	DI RNA
BYDV	Bacterial ribonuclease III
Groundnut rosette virus	Satellite RNA
Wheat bunt	KP4 virus infecting *U. maydis*
Rice blast and bacterial blight in rice, Fusarium head blight (FHB) and Fusarium seedling blight, *Phytophthora parasitica* var. *nicotianae*, crown gall of tomato, *pea early browning virus*, *brome mosaic virus*, *bean pod mottle virus*	RNAi

for increasing resistance levels of plants. For these strategies, the concept of "pathogen-derived resistance" (PDR) has been of most importance. The concept of PDR was encapsulated by Sanford and Johnston (1985) who suggested that the transgenic expression of pathogen sequences might interfere with the pathogen. Deployment of host plant resistance is the most effective approach for the control of seed-borne phytopathogens. Disease resistance genes could be sourced from plant pathogens themselves, e.g. coat protein-mediated plant viral resistance and with toxin inactivating protein-mediated bacterial resistance. Many successful examples of PDR are given in Table 24.4.

PDR for viral disease can normally be explained by the virus-derived transgene interfering with essential steps in the life cycle of the infecting virus. Potential application of PDR was first reported by Abel et al. (1986) who demonstrated that transgenic expression of TMV coat protein gene in tobacco produced resistance against TMV. Effectiveness of this approach was extended by the use of other viral genes like replicase gene, defective movement protein gene, viral helper component and viral protease gene, sequences of defective interfering nucleic acids, sequences incapable of encoding proteins, ameliorating satellite RNA sequences and viral nucleic acid (RNA or DNA) mediated, etc.

There are two basic molecular PDR mechanisms: protein-based resistance and nucleic acid-mediated resistance.

(a) *Protein-Based Resistance*

Coat Protein-Mediated Resistance (CPMR) CPMR refers to the resistance caused by the expression of the viral coat protein gene in transgenic plants. Coat protein gene is most widely used transgene to generate viral resistance and is based on the conventional phenomenon of "cross-protection". *Cross-protection* is the term used for the phenomenon that a plant, when first inoculated with a mild strain of a given virus, becomes protected against infection with a second, more severe strain of this virus. Accumulation of the CP gene in transgenic plants has been shown to confer resistance to infection or disease development by the virus from which the CP gene is derived and by related viruses. The approach of CPMR has also been shown to be commercially applicable. In 1998, "Rainbow" and "SunUp" transgenic virus-resistant papaya cultivars proved new hope for control of *Papaya ringspot virus* (PRSV) in Hawaii and are now widely planted and have helped to save the papaya industry from devastation. Transgenic papaya has also been developed for countries, such as Thailand, Jamaica, Brazil and Venezuela (Gonsalves 2004). The viruses include tobacco mosaic, potato virus X and Y and cucumber mosaic along with papaya ringspot in the crops like tomato, cucumber, tobacco and papaya.

Replicase Mediated Transformation of a plant with a DNA sequence derived from a gene encoding a viral replicase can endow it with a high level of resistance to the virus. Genetically engineered plants expressing either intact or mutant forms of the virus-encoded replicase subunit are resistant to infection by the virus from which the transgene was obtained. It is considered that expression of replicase-derived sequences at the protein level interferes with normal functioning and/or assembly of viral replicase enzyme complexes leading to a general, marked inhibition of viral replication in the cells of these transgenic plants (Carr and Zaitlin 1993; Carr et al. 1994). Replicase-mediated resistance as a PDR concept was first applied as encoding viral RNA- dependent RNA polymerases (RdRps) for TMV and first reported by Golemboski et al. (1990). For example, tobacco plants transformed with a gene encoding a truncated cucumber mosaic virus (CMV) 2a replicase protein are resistant to systemic CMV disease. To date, plants have been made resistant to *Tobacco mosaic virus*, *Pea early browning virus*, *Cucumber mosaic virus*, *Potato virus X* and *Potato leaf roll virus* (PLRV).

Movement Protein-Mediated Resistance Movement proteins (MPs) are encoded by plant viruses and enable infections to spread between adjacent cells (local spread) and systemically (Carrington et al. 1996). An interesting and potentially useful attribute of MP-mediated protection is the broad-spectrum efficacy of the resistance mechanism. This kind of resistance is generally requiring the expression of the dysfunctional protein and thought to be the result of competition between wild-type virus-encoded MPs and preformed, dysfunctional transgene-encoded proteins that disrupt the viral transfer system through plasmodesmata (Lapidot et al. 1993; Tacke et al. 1996; Seppanen et al. 1997).

(b) *Nucleic Acid-Mediated Resistance*

Expression of viral coat protein (CP) and replicase in plants yields protection to the homologous virus. A variety of PDR strategies involve the expression of genes encoding nucleic acids that lack the capacity to encode proteins. RNA-mediated resistance proved to be specific for the virus from which the transgene was derived, while protein-mediated resistance had an effect also on other viruses related to the one used as a source for transformation. As for protein expression levels, no direct correlation could be established between RNA expression levels of the transgene and the levels of resistance against virus infection. One of the earliest approaches was the expression of antisense RNA sequences to reduce the replication of RNA viruses which is as follows.

Antisense RNA and RNA Interference To date, the most well-known type of RNA-mediated resistance is RNA suppression via antisense RNA or RNA interference; these are processes that describe the post-transcriptional destruction of viral RNA also known as gene silencing (Baulcombe 1994). RNA silencing is a key regulator of gene expression which plays a major role in balancing by dynamically linking developmental programmes and environmental responses to gene expression changes through transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS). In plants, these pathways also function directly in host defence against viruses by targeting viral RNA for degradation (Pumplin and Voinnet 2013). In plants, three basic processes can be distinguished: post-transcriptional gene silencing (PTGS) mediated by small interfering RNAs (siRNAs), silencing mediated by microRNAs (miRNAs) and transcriptional gene silencing (TGS) mediated by siRNA-directed methylation of DNA and histone proteins. The main key components of these RNA silencing pathways have been shown to have an important protective role against invading viral pathogens (Bologna and Voinnet 2014). When RNA virus infects plants during replication, it forms dsRNA and then it is degraded into 21–24 nucleotide dsRNA fragments, known as small interfering RNAs (siRNAs). These siRNAs are incorporated into a complex called RISC (RNAi silencing complex) which degrades mRNAs that are complementary to the single-stranded siRNA. A general mechanism of endogenous RNA silencing pathway is given in Fig. 24.3.

Ribozymes These are catalytic RNAs that can cleave at specific sites in complementary target RNAs. Some viroids, virusoids and satellite RNAs perform self-cleavage reactions. Mostly, the cleavage site consists of a consensus structure, called the "hammerhead" motif. Huttner et al. (2001) used ribozyme genes to protect melon plants against two potyviruses: *Watermelon mosaic virus* 2 (WMV2) and *Zucchini yellow mosaic virus* (ZYMV).

By Satellites In satellite viruses, the satellite nucleic acid codes for its own coat protein. In satellite RNAs or DNAs, the nucleic acid becomes packaged in protein shells made from coat protein of the helper virus. Replication of the satellite inter-

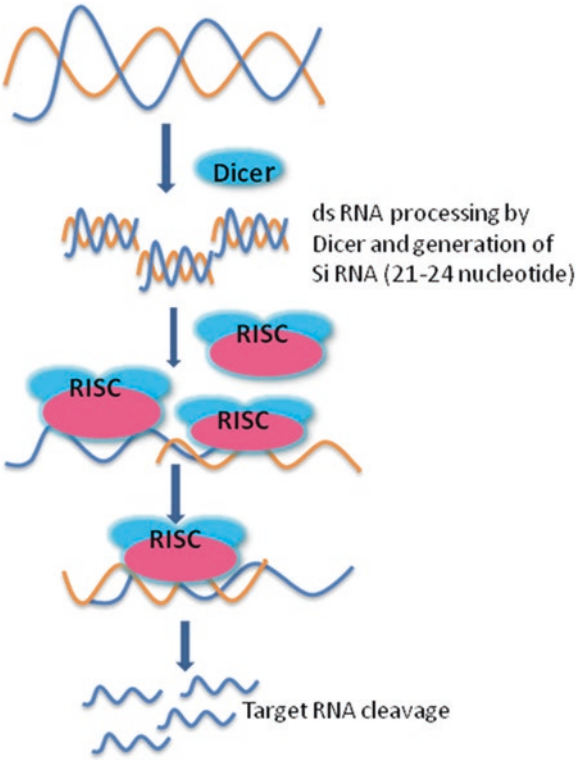

Fig. 24.3 RNA silencing mechanism steps. RNA silencing is initially triggered by double-stranded RNA (dsRNA) which can arise endogenously from transcription. These dsRNAs are processed by ribonuclease III-type DICER-like (DCL) enzymes into small RNAs (sRNAs), 21–24 nucleotides in length, e.g. miRNA and siRNA, which are incorporated into an RNA-induced cytoplasmic silencing complex (RISC). Once integrated into the RISC can target siRNA- or miRNA-complementary mRNAs and induce their post-transcriptional gene silencing (PTGS) by endonucleolytic cleavage ("slicing") or translational repression

feres with replication of the helper. The mechanism of satellite RNA (Sat-RNA)-mediated resistance is attenuation of virus disease as a result of competition between satellite RNAs and their helper viral RNAs for replication. In many cases, they are amplified at the expense of the parent virus, ameliorating the symptoms induced by that virus. For example, transformation of *Nicotiana benthamiana* with full-length sequences of a mild variant of the groundnut rosette virus (GRV) satellite RNA (sat-RNA) yielded plants that did not produce symptoms when inoculated with GRV and a virulent sat-RNA (Taliansky et al. 1998).

Defective Interfering Nucleic Acids Truncated, and often rearranged, versions of genomic viral nucleic acids are associated with many plant RNA viruses and some plant DNA viruses. These subviral nucleic acids (RNA or DNA) are termed defective interfering (DI) which depend on their parent virus for replication. These RNAs or DNAs are called "defective" because they have lost the capacity to code for the necessary viral proteins for independent replication and thus are defective in the absence of the parent virus. The first DI-RNAs associated with a plant virus identified were derived from *Tomato bushy stunt virus.* DI-RNAs can be synthesized by many processes, e.g. replicase-driven template switching, forced template switching, RNA breakage and ligation mechanism (Pathak and Nagy 2009). Dl can

attenuate the disease symptoms of their helper virus by competition for viral and host resources and can trigger gene silencing response in host.

For Bacterial and Fungal Disease Transgenic resistance to fungi and bacteria has generally been more difficult than viral disease. There are many transgenes, e.g. chitinases, glucanase, etc., of fungal and bacterial origin expressing in transgenic plants for degradation of fungal and bacterial cell wall material that showed enhanced resistance to fungal and bacterial disease in many studies (Table 24.4).

More than 30 bacterial and many fungal avirulence genes which were used to create and understand the signalling and resistance have been cloned and characterized (Leach and White 1996). For example, *Fusarium graminearum* protects itself through expressing a protein that rapidly converts non-secreted DON to a less active acetylated form through the action of trichothecene 3-O-acetyltransferase (tri10). Overexpression of *FusariumTri10* gene in transgenic wheat resulted in reduction in fungal infection during greenhouse trials and subsequently resulted in less toxin contamination in grains of rice and barley inoculated with *F. graminearum* (Okubara et al. 2002; Manoharan et al. 2006).

24.3.3.2 Host-Derived Resistance

An alternative use of pathogen-derived genes for introduction of resistance is to transform plant by inducing or overexpressing their own or other plant genes conferring resistance into distinct plant species or varieties. Plants also harbour an enormous number of disease resistance genes such as those encoding R genes; pathogenesis-related (PR) proteins; hydrolytic enzymes; antimicrobial compounds, e.g. proteins, phytoalexins and neutralizing virulence products; plant ribosome-inactivating proteins and other peptides, etc. (Fig. 24.4)

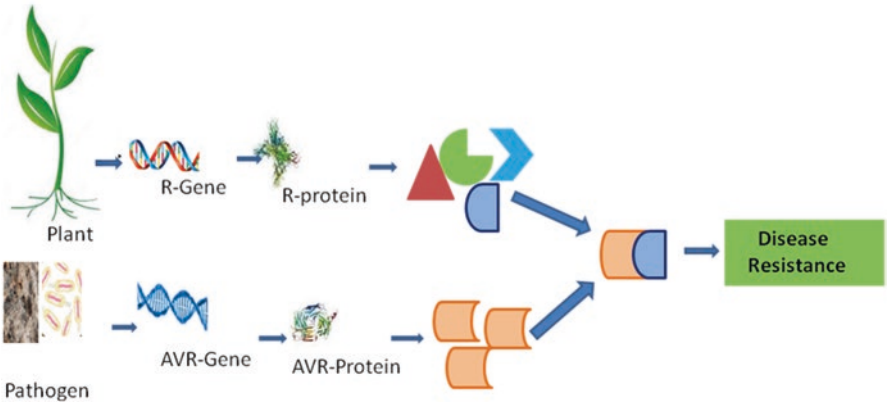

Fig. 24.4 R gene-mediated resistance

Table 24.5 List of overexpressed gene in cereals

Plants	Pathogen	Overexpressive gene/QTL	References
Rice	Blast + bacterial blight	BSR1	Maeda et al. (2016)
	Blast	Pikh, *OsCPK4*	Bundo and Coca (2016) and Azizi et al. (2016)
	Bacterial blight and sheath blight	*Os2H16*	Li et al. (2013b)
Wheat	*Fusarium* head blight	TaWRKY45	Bahrini et al. (2011)
		α-1-purothionin, thaumatin-like protein 1 (tlp-1) and β-1,3-glucanase; class II chitinase	Mackintosh et al. (2006) and Shin et al. (2008)
	Spot blotch	TaPIEP1	Dong et al. (2010)

Overexpression of Defence Genes A number of identified genes or gene variants provide resistance to specific pathogens. Researchers have discovered that overexpression of these genes likely improves resistance to a broad spectrum of pathogens. Wang et al. (2013) have demonstrated that overexpression of certain Elongator gene family (ELP1, ELP2, ELP3, ELP4, ELP5 and ELP6) enhances plant disease resistance in *Arabidopsis*, strawberry, citrus and tomato to multiple pathogens, and they have also found that the number of spores of powdery mildew pathogen was 90–96% lower in transgenic plants than in the control plants. Similarly, transgenic wheat lines carrying a barley seed class II chitinase exhibited enhanced resistance to powdery mildew (Bliffeld et al. 1999; Oldach et al. 2001).

Overexpression of the *NPR1/NIM1* gene in *Arabidopsis* leads to enhanced resistance against *Pseudomonas syringae* and *Peronospora parasitica*, with no obvious detrimental effects on plant growth or development (Cao et al. 1998). Mackintosh et al. (2007) developed a transgenic wheat overexpressing ß-1,3-glucanase gene along with a-1-purothionin and thaumatin-like protein 1 (tlp-1) gene. The resultant transgenic wheat lines were tested against *F. graminearum* infection; overexpression of these genes enhanced the fungal resistance in wheat. More examples are listed in Table 24.5.

Use of Resistance Genes Wild relatives of crop species, the major source of resistance genes, could be used to engineer ecological disease control. However, breeding R genes into crop lines often requires long timelines of 5–15 years to break linkage between R genes and deleterious alleles. Several cloned R genes were available; it would be possible to pyramid these R genes in a crop, which might provide more durable resistance (McDonald and Linde 2002).

For example, to date nearly 100 blast resistance (*R*) genes and over 350 quantitative trait loci (QTLs) have been identified, of which 27 have been cloned and characterized, i.e. *Pb1*, *Pia*, *Pik-m*, *Pik-p*, *Pi56*, *Pi63* and *PiCO39* (Devanna et al. 2014),

Table 24.6 Transgene used for host-derived resistance for seed-borne diseases

Crop name	Pathogen	Transgene	References
Wheat (*Triticum aestivum*)	*Blumeria graminis* f. sp. *tritici*	9f-2.8 (oxalate oxidase) and TaPERO (peroxidase) from wheat	Altpeter et al. (2005)
	Fusarium graminearum	NPR1 from *Arabidopsis thaliana*	Makandar et al. (2006)
Rice (*Oryza sativa*)	*Xanthomonas oryzae* pv. *oryzicola*	Rxo1 (NBS-LRR) from maize (*Zea mays*)	Zhao et al. (2005)
	Xanthomonas oryzae pv. *oryzae*	Xa21 (NBS-LRR) from rice (*Oryza longistaminata*)	Wang et al. (1996), Zhang et al. (1998), and Zhai et al. (2002)
Maize (*Zea mays*)	*Fusarium graminearum*	Gfzhd101 from *Clonostachys rosea*	Igawa et al. (2007)
Tomato (*Lycopersicon esculentum*)	*Pseudomonas syringae* pv. *tomato*	Pto from *Lycopersicon pimpinellifolium*	Tang et al. (1999)
Potato (*Solanum tuberosum*)	*Phytophthora infestans*	Rpi blb2 (NB LRR) from *Solanum bulbocastanum*	van der Vossen et al. (2003, 2005)
Cucumber (*Cucumis sativus*)	*Cucumber mosaic virus* (CMV)	CMV *2b* gene (suppression of host RNA silencing and viral virulence)	Jacquemond (2012)
Soybean (*Glycine max*)	*Soybean mosaic virus* (SMV)	3′-UTR (transforming with the coat protein gene and the 3′-UTR from SMV)	Wang et al. (2001)
Soybean (*Glycine max*)	*Bean pod mottle virus*	GmFAD3 silencing	Singh et al. (2011)
Pea (*Pisum sativum*)	*Alfalfa mosaic virus* (AMV)	AMV CP sequences (NZ1 (Lincoln) and NZ34	Timmerman-Vaughan et al. (2000)
Pea (*Pisum sativum*)	Pea seed-borne mosaic potyvirus (PSbMV)	Replicase (NIb), virus-induced gene silencing	Jones et al. (1998)

and at least 38 BLB R genes (both dominant and recessive) from Xa1 to Xa38 have been identified (Bhasin et al. 2012; Kumar et al. 2012; Khan et al. 2014). An elite *indica* rice variety, "IR72", was transformed with a cloned gene, *Xa21*, which was found resistant to bacterial blight (Tu et al. 1998). A number of rice varieties including IR64, IR72, IR50, CO39, Pusa Basmati-1, IR68899B, MH63, BPY5204 and some Chinese lines were genetically engineered to contain the gene. A list of genotypes used for resistance of seed-borne diseases is shown in Table 24.6.

These were two chitinase genes (RCH10, RAC22) from rice, a glucanase gene (β-Glu) from alfalfa and a ribosome-inactivating protein gene (β-RIP) from barley (Zhu et al. 2007). Transgenic plants and their progenies thus produced were found to possess significant resistance to rice blast disease (Fig. 24.5).

Plant Resistance Gene Analogues (RGAs) Plant RGAs are a huge cluster of potential *R* genes that have conserved domains and structural segments that play specific roles in host-pathogen interactions. Efficient mechanisms have been developed by

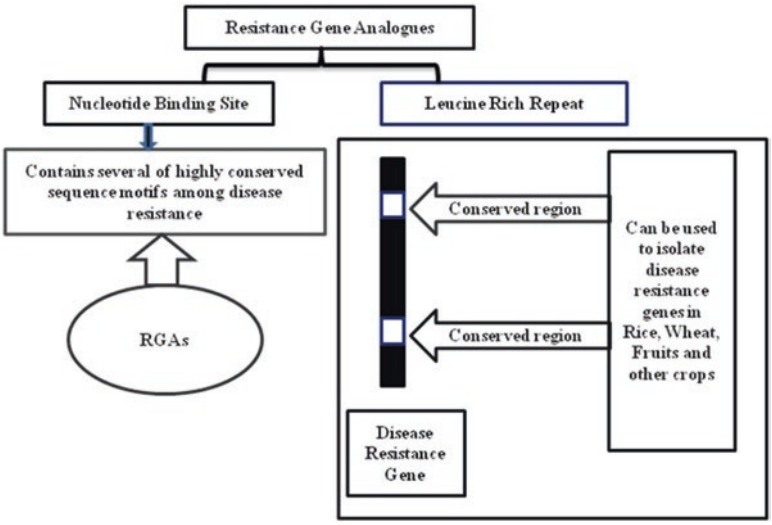

Fig. 24.5 Schematic representation of RGAs (resistance gene analogues)

plants to identify and react to infections caused by pathogens. Pathogens' resistance is governed by conserved domains and motifs that are present in resistance gene analogues (RGAs). Well-known RGAs are nucleotide binding site leucine-rich repeats, receptor-like kinases and receptor-like proteins. RGAs can be noticed using bioinformatics tools based on their conserved structural segments. A large number of RGAs has been recognized from sequenced plant genomes. High-density RGA genetic maps are helpful for designing investigative markers and identifying QTLs/markers associated with plant disease resistance. Recent advances in structure and functional mechanisms of resistance gene analogues (RGAs) and nucleotide binding site leucine-rich repeat (NBS-LRR) family, receptor-like kinase (RLK) and receptor-like protein (RLP) families, bioinformatics approaches for RGA identification and characterization, genome-wide identification and characterization of RGAS, genome organization of RGAs and applications of RGAs have been described (Sekhwal et al. 2015). RGAs candidates for disease resistance, genome-wide association study (GWAS) with mapped rgas helps co-localization of QTL to resistance genes and rga mapping in plants with limited genome information is worth reading. RGAs have been successfully used in a wide range of crop plants for identification of disease resistance genes (Table 24.7).

Induced Expression of Antifungal, Antibacterial and Antiviral Compounds Several characteristic features of pathogen structure and cell metabolism represent potential targets for inhibitory agents. The constitutive or pathogen-inducible expression of genes encoding proteins with antipathogenic capacity in transgenic plants may enhance resistance to damage in plants. Depending upon the mechanism of plant disease resistance, the transgenic plants

Table 24.7 Crops where RGAs have been successfully used

Crop	References
Arabidopsis thaliana (*Arabidopsis*)	Meyers et al. (2003), Shiu et al. (2004), Fritz-Laylin et al. (2005), and Yu et al. (2014)
Vitis vinifera (grape)	Yu et al. (2014) and Yang et al. (2008)
Solanum lycopersicum (tomato)	Andolfo et al. (2014)
Carica papaya (papaya)	Ming et al. (2008)
Cucumis sativus (cucumber)	Yang et al. (2013)
Solanum tuberosum (potato)	Lozano et al. (2012)
Medicago truncatula (*Medicago*)	Yu et al. (2014)
Gossypium raimondii (cotton)	Wei et al. (2013) and Chen et al. (2015)
Brassica rapa (Chinese cabbage)	Yu et al. (2014)
Brassica oleracea (cabbage)	Yu et al. (2014)
Fragaria vesca (strawberry)	Li et al. (2013a)
Malus domestica (apple)	Kobe and Deisenhofer (1994)
Lotus japonicus (lotus)	Li et al. (2010)
Theobroma cacao (cocoa)	Yu et al. (2014)
Physcomitrella patens (moss)	O'Toole et al. (2008) and Xue et al. (2012)
Oryza sativa (rice)	Zhou et al. (2004), Fritz-Laylin et al. (2005), Dardick et al. (2007), O'Toole et al. (2008), and Singh et al. (2015)
Triticum aestivum (wheat)	Bouktila et al. (2014)
Zea mays (maize)	Cheng et al. (2012) and Wang et al. (2014)
Sorghum bicolor (sorghum)	Guo et al. (2011)
Hordeum vulgare (barley)	Gu et al. (2015)
Brachypodium distachyon (*Brachypodium*)	Tan and Wu (2012) and Gu et al. (2015)

have expressing genes for disease resistance for a number of antimicrobial compounds. Plant proteins that are produced in response to pathogen invasion and hinder growth, differentiation and multiplication of pathogens are called pathogenesis-related proteins (PR proteins). So far 17 families of PR protein have been identified. The constitutive or inducible expression of genes encoding PR proteins in transgenic plants enhanced resistance. Among the PR proteins hydrolytic enzymes (chitinase and glucanase), osmotins, thionins and defensins are especially important. For instance, the ß-1,3-glucanases are proteins believed to be part of the constitutive and induced defence system of plants against fungal infection (Sela-Buurlage et al. 1993).

Invoking the hypersensitive reaction, plant varieties that are naturally resistant to specific types of fungal diseases are often programmed for quick death of individual cells at the site of fungal infection. Phytopathogens often produce toxins during plant infection and these toxins may act as virulence factors (Desjardins and Hohn 1997).

Inactivation of these toxins or their targets in plants has led to enhanced disease resistance. Promising avenues for disease control in these systems may include engineering insensitive variants of toxin targets in the plant, expression of toxin-inactivating enzymes (Kimura et al. 1998) or blocking entry of the toxin into the plant cell.

Phytoalexins are low molecular weight secondary metabolites with antimicrobial activities produced in plants after pathogen attack. So, overexpression of such genes in unrelated plants may be fruitful to achieve the goal. Expression of antimicrobial peptides or metabolites or lytic peptides are small proteins with antimicrobial activity that appear to be major components of the antimicrobial defence system. Lytic antimicrobial peptides are generally 23–39 amino acid sequences.

24.4 Other Applications

24.4.1 Plantibodies

It has long been assumed that plants could not produce antibodies. Gene engineering achievements have opened an opportunity to develop transgenic plants where molecules of antibodies or their functional fragments are expressed. Plant-produced antibodies, namely, plantibodies, were first demonstrated by Hiatt et al. (2001).

Recent confirmation of the biological activity of an anti-phytochrome mouse monoclonal single-chain F in transgenic tobacco supports this overall strategy. Transgenic tobacco plants expressing a single-chain variable region antibody derived from the monoclonal antibody 3–17 showed resistance to *Potato virus Y* and *Clover yellow vein virus* (ClYVV). It suggests that one single-chain construct can be used to protect plants from distinct potyviruses (Xiao et al. 2000). Another example is the expression of a scPv antibody specific for the stolbur phytoplasma major membrane protein, which enhanced plant resistance to phytoplasma pathogens (Le Gall et al. 1998). Peschen et al. (2004) have expressed fusion proteins comprising chicken-derived single-chain antibody fragments against *F. graminearum* linked to antifungal peptides in *Arabidopsis thaliana*. When the transgenic plants were challenged with *F. oxysporum*, a high level of protection was obtained, whereas expression of either antibody or antifungal peptides alone resulted in moderate levels of protection only.

24.5 Role of Biotechnological Tools or Technique in Quarantine of Seed-Borne Diseases

Quarantine is an essential strategy for seed-borne disease management. It covers all regulatory actions taken to exclude pathogens from a site, area, country or group of countries. During quarantine, plants are monitored for disease symptoms and signs of disease infestation and correctly identify it. Although some diseases can be diagnosed quickly by visual examination, some diseases have no external symptoms and require laboratory testing for diagnosis, for example, loose smut of wheat. These laboratory procedures may take days or even weeks to complete and are, in some

cases, relatively insensitive. Delays are frustrating when a quick diagnosis is needed so that appropriate quarantine measures may be taken to prevent introduction of disease in new area, especially when seed is infected with high-risk pathogen. Fortunately, as the result of advances in biotechnology, new tools and techniques are becoming available that will complement or replace time-consuming laboratory procedures with high accuracy.

Now biotechnology has played an integral role to deal with emerging challenges in the quarantine of seed-borne diseases and used as a successful tool for increasing security. Improvements in molecular and serological techniques have allowed the development of sensitive, highly specific tests for many important seed-borne pathogens. There are many techniques routinely based on immunological and DNA technique given in Table 24.8. These tests are now routinely applied in quarantine for exerting disease surveillance and quality assurances of seeds. Recently, biochips composed of densely packed probe microarrays have brought dramatic changes and are very flexible and robust and have the potential to increase diagnostic throughput while simultaneously reducing unit cost. Both nucleic acid-based and antibody-based microarrays are being developed. Microarray-based techniques such as high-throughput multiplexed ELISA are being adapted for disease diagnosis (Martin et al. 2000), for example, Karnal bunt (KB) of wheat which has become a major disease of economic importance and an important issue in the international wheat trade and other many seed-borne viral diseases. Recently KB genomes have been decoded by ICAR-IIWBR, Karnal (Sharma et al. 2016). Development of specific markers and identification of effectors will help in field diagnostic kit quickly (Sharma et al. 2018).

24.6 Role of Biotechnology in Agrochemicals for Seed-Borne Diseases

Seed treatment is an effective tool for combating the negative impacts of seed-borne diseases. It protects germinating seeds and seedlings against seed-borne as well as soil-borne pathogens to prevent plant disease epidemics. The seed treatment market is projected to reach a value of USD 9.82 billion by 2021 in which 35% of fungicides will be used as seed treatment. Furthermore, seed treatments can be useful in reducing the amounts of pesticides required to manage a disease, because effective seed treatments can eliminate the need for foliar application of fungicides later in the season. The current methods used to find and develop new pesticides are sophisticated and complex. Development, testing and registration can typically take 8–12 years and cost over $50 million for each pesticide. Another problem is development of resistance because many of the known target sites are no longer available; chemicals that contain novel modes of action are always needed. Currently used fungicides have been shown to be effective over the years, but recently many pathogens, e.g. wheat-infecting *Fusarium* spp., have developed resistance.

Fungicides currently used in agriculture only address a limited number of molecular targets. Thus, the discovery of novel fungicides with novel modes of action would represent a breakthrough in chemical plant protection, so new target site selection is

Table 24.8 Biotechnological technique in quarantine of seed-borne diseases

S. no	Biotechnological technique or tool	Explanations	Examples
1	Polymerase chain reaction (PCR) and its variant RT-PCR, BIO-PCR, multiplex PCR, etc.	It enables amplification and multiplication, up to a manifold, of the target sequence of DNA, e.g. ITS region and α-tubulin sequences in fungi by specific markers	seed-borne fungi of common bean (Mancini et al. 2016)
2	Loop-mediated isothermal amplification (LAMP)	Amplifies a few copies of target DNA with high specificity, efficiency and rapidity under isothermal conditions, using a set of four specially designed primers and a DNA polymerase with strand displacement activity	*Tilletia indica* (Gao et al. 2016)
3	DNA barcoding	Uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species, i.e. ITS cytochrome c oxidase subunit I (COI), nuclear large ribosomal subunit, nuclear small ribosomal subunit, largest and second largest subunit of RNA polymerase II, elongation factor 1-α, small subunit of the mitochondrial ribosomal operon, BenA (β-tubulin), actin, chitin synthase, calmodulin and heat shock protein 90	*Tilletia horrida* causing rice kernel smut (Chen et al. 2016)
4	Next-generation sequencing (NGS)	The entire genomic information (DNA or RNA) is sequenced	*Tomato necrotic stunt virus* 1 (Singh-Gasson et al. 1999)
5	Microarray	Glass slide spotted with thousands of probes. Each probe is a piece of DNA sequence similar to the pathogen sequences. The complementary binding of probes to the sequences will emit fluorescent signals detectable by a scanner	DNA microarrays of the rice blast fungus, *Magnaporthe grisea*, and rice plant on one chip (www.agilent.com/chem/agbiotech)
6	Molecular beacon (MB)	MB is single-stranded nucleic acid molecules with a stem-loop conformation loop portion consisting of probe sequences that are complementary to the target sequences of choice	*Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) of orchid (Eun and Wong 2000)
7	Nanodiagnostics, i.e. nano-biotransducer or biosensors	Nanomolecular diagnostic is the use of nanobiotechnology to diagnose plant disease	*Xanthomonas axonopodis* pv. *vesicatoria* that cause bacterial spot diseases in Solanaceae (Yao et al. 2009)

(continued)

Table 24.8 (continued)

S. no	Biotechnological technique or tool	Explanations	Examples
8	Fluorescence in situ hybridization (FISH)	The method detects specific sequences of nucleic acids by hybridization of fluorescently labelled probes to complementary target sequences within intact cells, i.e. bacterial, fungal and viral pathogens	*Cucumber green mottle mosaic virus* (Shargil et al. 2015)
9	Enzyme-linked immunosorbent assay (ELISA) and its variants	Identification of diseases based on antibodies and colour change in the assay	Generally, for detection of seed-borne fungal, viral and bacterial disease
10	Nano-gold-based lateral flow immune-dipstick test	Surface plasmon resonance (SPR)-based immuno-sensing system	Detection of fungal teliospores of Karnal bunt (KB) disease of wheat incited by *Tilletia indica* (Singh et al. 2012)
11	Polymerase chain reaction (PCR) technique	For accurate identification of teliospores of the Karnal bunt pathogen, *Tilletia indica*	Karnal bunt pathogen (Bonde et al. 1997; Sharma et al. 2018)
12	Micropropagation	Screening for pathogen and contaminating microorganisms in micropropagation	For the rapid diagnosis of plant pathogens (Cassells 1992)
13	Improved PCR method	Utilizing TaqMan for the detection and differentiation of *Tilletia indica*	Causal organism of Karnal bunt of wheat and a related grass smut (Frederick et al. 1998)

necessary that allows the development of new fungicides (Pandey and Sharma 2018). By combining both genomic data and molecular modelling to increase the efficiency of discovery for new chemical compounds with safety, lower costs and low environmental impact as whole genome sequence of pathogens are getting available (Sharma et al. 2016). So, we can select protein targets from the genome to develop chemical compounds against a pathogenic organism. For example, Martin et al. (2000) identified three major new target sites: endoglucanase-5, a trichodiene synthase and a 24-sterol C-methyltransferase using molecular structural-based approach by genomic analysis. A new compound has to be highly efficacious and environmentally acceptable. In the past, screening took many years but current method of screening for new pesticides starts with high-throughput screens (HTS) that can handle hundreds of thousands of compounds per year. These screens include miniaturized systems in which whole organisms are grown on microtiter plates or other systems but require microgram or nanogram quantities of material for tests, which can be run in a minimum of space and time (Jansson et al. 1997), e.g. new classes of fungicides, viz. strobilurins, phenylpyrroles, anilinopyrimidines and phenoxyquinolines, which have been identified through the development of HTS comprising of target enzyme sites or

cell-based assays. Besides this use of in vitro and in vivo assays against molecular targets for the evaluation of chemicals is also advocated. For example, an immunological ciELISA was developed to determine the potential inhibitor of acetyl coenzyme-A carboxylase (ACCase)-specific monoclonal antibodies as screening tools. Proteomic information about fungal, bacterial as well as viral plant pathogens may be an incentive to the development of new environment-friendly fungicides. Moreover, bioinformatics may be of help in predicting a protein as a fungicide. Biosynthetic fungicide design that is disease-associated target oriented has been established as a new focus in fungicide development nowadays (Acero et al. 2011). The use of modified natural compounds provides a potential species-specific method of controlling plant pathogens by the specific inhibition of those proteins involved in the infection cycle (Pinedo et al. 2008). The use of these compounds minimizes the environmental impact if they are biodegradable, possesses high specificity and has the further advantage of poor penetration into the food chain. In short, such an application of chemogenomics to protein targets is named "chemo-proteomics" although a more explicit definition is target-related affinity profiling (TRAP), defined as the use of biology to inform chemistry (Beroza et al. 2002).

24.7 Conclusion

Biotechnology brings new hopes for overcoming various constraints associated with conventional control methods and breeding varieties for durable resistance by pyramiding genetically engineered resistance over intrinsic plant resistance. The primary benefit of deploying resistance genes in transgenic technology is its ability to overcome the fertility restraints for the dispersal of genes originating from a different species. Therefore, elucidation of biochemical functions of pathogen avirulence proteins and plant R proteins and control of the defence signal transduction pathways in the host leading to the expression of resistance are warranted. Application of biotechnological tools for disease resistance could not only help us to better understand the plant defence signalling; it could reveal novel insights on the interactions between these signalling pathways and other plant processes. Even though the progress towards the overall plant defence mechanism studies is going on at a considerable pace, attempt to manipulate both Avr and R gene sequences would still be imprudent to expect a great breakthrough in impervious broad-spectrum and durable disease resistance. As we know, managing disease through seed health testing could become more important as well as a new way of managing it and in this regard latest developments in biotechnology have a great impact on seed health assays. But more research is needed on the biology of seed infection. New strategies are constantly being developed and several new approaches have emerged in the past few years. Some of these have proved to be effective in either reducing seed-borne or seed-transmitted phytopathogens and would appear to be useful tools for the management of seed-borne diseases as an adjunct to seed health perspective.

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Management of Seed-Borne Diseases: An Integrated Approach

25

Anuja Gupta and Ravindra Kumar

Abstract

Productivity and sustainability in agriculture can be achieved either by increasing crop production through the use of high-yielding crop varieties or by avoiding crop failures mainly due to pests and diseases. High-yielding cultivars are often susceptible to one or more diseases. The basic need therefore is availability of good quality healthy seed/planting materials. Seeds are known to be carriers of a large number of microorganisms. Since about 90% of the crops are grown through seeds, they are also a potent source for dissemination of various diseases and survival of pathogens from season to season. Healthy seed can be obtained through appropriate certification schemes or by effective seed treatments, but it is difficult to restrict seed-transmitted diseases, which are also soil-borne, or perpetuate on plant residues. In order to reduce yield losses caused by diseases, farmers adopt calendar-based chemical spraying schedules rather than need-based sprayings, which lead to chemical residues in the produce and development of resistance in the pathogens and disturb the natural fauna. Losses due to plant diseases are expected to have more critical influence on human being in the coming years than they have had in bygone years. An integrated approach is needed for the effective control of the diseases and production and maintenance of pathogen-free seed in the field and during storage.

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25.1 Introduction

The green revolution initiated in the late 1960s dramatically transformed the food security situation in India with steady increase in the domestic food grain production and shifting the status of the country from food scarcity to food security. Food grain production in the country increased from 50 MT in 1950–1951 to record production of 284.83 MT in 2017–2018. However, to meet the growing demands for the ever-increasing population which is likely to be 1.7 billion by 2050, we need to double our food production. Seed is recognized as the cheapest yet most critical input for increasing agricultural productivity. Use of good quality seed alone can result in 15–20% increase in crop yields. Production of healthy seed is undertaken in areas and crop seasons having negligible disease incidence. The seed plots of breeder, foundation, and certified categories are monitored at pre-flowering, flowering, and mature crop growth stages against designated seed-borne diseases, and the fields are rejected which fail to meet minimum certification standards of field infection. The incidence of the diseases varies with the regions, crop varieties, and the environment. New races, mating types, and fungicide-resistant strains of pathogens are also introduced along with the seed/planting materials across the countries. Thus, there is a strong need for development of integrated approach for management of diseases to sustain crop production.

The idea of integrated disease management (IDM) is acquired from integrated pest management (IPM) concept which was initiated by the entomologists of the University of California, USA. This concept was based on "integrated control" which they defined it as "applied pest control which combines and integrates biological and chemical control and using chemical control only if necessary and, in a way, which was least disruptive to biological control" (Razdan and Gupta 2009). The problems of pest resistance to insecticides and the ecological damage recognized due to extensive use of pesticides in the late 1950s and early 1960s have realized the need of the concept of IPM. Therefore, IDM comprise of timely application of a combination of strategies and tactics. These may include proper selection and preparation of area, cultivation of resistant cultivars, use of disease-free certified seed, altering sowing/planting practices as time of sowing and plant spacings, agricultural practices as irrigation, crop density, application of agrochemicals, etc. Moreover, monitoring of environmental factors (temperature, moisture, soil pH, nutrients, etc.), disease forecasting, and establishing economic thresholds are also important factors for development of a management schedule. These measures should be applied in a coordinated, integrated, and harmonized manner to maximize the benefits of each component. The principles of plant disease management should always be based on the integration of basic concepts such as avoidance, exclusion, eradication, protection, resistance, and therapy or curative control. Specialized control practices for particular diseases are not included as they are variable and change with the crop, area, disease pressure, etc.

25.2 Integrated Disease Management Strategies

The concept of disease management should include management of disease at an economic threshold level since complete elimination of the diseases results in resurgence of target pathogen and development of resistant strains. This is accomplished by lowering the inoculum level of the pathogens and/or inhibiting their growth by making unsuitable growth conditions. Integrated disease control strategy combines genetical, cultural, chemical, and biological methods in a compatible manner resulting in the growth of healthy plants. Plant diseases caused by fungi, bacteria, viruses, and nematodes may affect the plants from seedling to fruiting stages causing significant yield losses. The present chapter is a comprehensive approach of various methodologies and principles for successful plant disease management. Various strategies, tactics, and techniques used in disease management can be grouped under two principles, prevention (prophylactic) and therapy or curative (treatment or cure). Prevention comprises of practices undertaken for management of plant diseases prior to establishment of infection, whereas curative action refers to practices undertaken post establishment of the pathogen. Application of quarantine measures helps in prevention of a disease in any region, whereas physical or chemical treatment of seeds and vegetative propagative materials help in elimination of the established phytopathogens. For effective management of a disease, crop health surveillance involving monitoring of disease prevalence and severity in different areas along with the cropping system, meteorological data is also needed. Plant disease management practices rely on anticipating occurrence of disease and attacking vulnerable points in the disease cycle (i.e., weak links in the infection chain). Therefore, correct diagnosis of a disease is necessary to identify the pathogen, which is the real target of any disease management program. A thorough understanding of the disease cycle, including climatic and other environmental factors that influence the cycle, and cultural requirements of the host plant, is essential for effective management of any disease. Thus, it is essential to know the nature of the causal organism, its mode of penetration, method of spread, and survival or mode of perennation. It is comparatively easy to assess seeds for the associated pathogen and also to control them effectively. Seed health test has been considered as an important component of disease management strategy. In general, the four principal methods of controlling plant diseases are (1) exclusion, (2) eradication, (3) protection, and (4) immunization.

Spread of a disease in the field depends on the source of pathogen inoculum. In seed-borne diseases, the initial infected plants are more uniformly spread, but for soil-borne diseases, the infected plants may often appear in the clusters, and random spread may be observed in case of insect-transmitted diseases. Thus, for developing any strategy, it is important to know about the presence of a pathogen and its mode of perpetuation. For development of any management strategy, it is also important to be aware of the history of previous crops in the field and also previous weather data which might be helpful in prediction of any outbreak of a disease.

25.2.1 Exclusion

This principle is aimed at preventing introduction of new disease-causing agent (pathogen) from invading uninfected areas, region, farm, or planting material and avoiding contact between the pathogen and the crop. The idea for this strategy is based on the assumption that most pathogens require some means like host or non-host plant material or soil or packing material or containers or vectors to travel over long distances and their spread gets obstructed due to natural barriers like oceans, deserts, and mountains. Exclusion of a pathogen usually delays its entry and gives some time to plan its management strategy on its arrival. It was assumed that Karnal bunt disease of wheat which originated in India and is caused by a pathogen *Tilletia indica* would spread to Europe and the USA also. Hence, though preventive measures were taken to prevent its entry, it managed to reach the USA (Ykema et al. 1996), establishing a significant new origin on an existing trade route for possible entry into Europe and parts of the world (Sansford et al. 2008). Quarantine regulations, seed certification, crop inspections, etc. are some measures to prevent spread of the pathogens.

25.2.1.1 Quarantine Regulations

Quarantine can be defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion or prevention in spread of plant pests and diseases in uninfected areas. The introduction of new cultivars or breeding material is bound to be accompanied by introduction of new pathogens, some of which may become disastrous, whereas others may contribute to a less conspicuous but still important decline in the crop production. Spread of diseased material within the country must also be prevented. The quarantine laws were first enacted in the USA in 1912 and are known as Federal Quarantine Acts. In India, there are 26 plant quarantine and fumigation stations (PQ & FS) located at 10 international airports, 9 seaports, and 7 land frontiers. The import of seeds/planting materials is regulated as per provisions of The Plants, Fruits and Seed (Regulation of Import into India) Order, 1989 (PFS Order), issued under DIP (Destructive Insects and Pests) Act, 1914. The quarantine responsibility for plant genetic resources has been entrusted upon the National Bureau of Plant Genetic Resources, New Delhi, for agri-horticultural crops; Forest Research Institute, Dehradun, for forest plants; and Botanical Survey of India, Kolkata, for other plants of general economic importance.

A number of plant diseases have been introduced and got established in different countries (Table 25.1). Golden nematode (*Heterodera rostochiensis*) and wart (*Synchytrium endobioticum*) diseases of potato have been introduced in India from the European countries and are now well established in the Nilgiri hills and Darjeeling area, respectively. Fortunately, timely action has prevented the spread of wart disease to other parts of India. Some other interceptions include *Cryptosporella viticola* (*Phomopsis viticola*), *Diaporthe phaseolorum* (*Phomopsis sojae*), *Peronospora manshurica*, *Phoma lingam*, *Phoma betae*, *Agrobacterium tumefaciens*, etc. Certain viruses as *Bean yellow mosaic virus*, *Cowpea aphid borne mosaic*

Table 25.1 Introduction of some diseases in new regions of the world

Disease	Year	Introduced in	Introduced from
Late blight – potato	1830	Europe	S. America
Powdery mildew – grapes	1845 1910	England India	USA Sri Lanka
Bunchy top – banana	1940	India	Sri Lanka
Downy mildew – vine	1878	France	USA
Leaf rust – coffee	1879	India	Sri Lanka
Citrus canker – citrus	1907	USA	Asia
Paddy blast – rice	1918	India	S.E. Asia

virus, *Pea seed-borne mosaic virus*, *Soybean mosaic virus*, etc. have also been intercepted on imported legume seeds (Rana et al. 1993). Hence, it has become mandatory that the acquired germplasm accessions be grown in post entry quarantine net houses under constant vigil. The seedlings showing viral symptoms are uprooted, and further detection and identification of virus is conducted using a combination of tests as ELISA, electron microscopy, dot immunobinding assay, nucleic acid-based assays, etc., and harvest from only virus-free plants is released to the indentor.

The quarantine system still presents high risk and hazard from exotic pests and diseases. If a new and serious disease gets established in the country, various countries may put embargo on import of agricultural products (Rana et al. 1993). An importing country insists on zero tolerance levels against Karnal bunt of wheat for fear of introducing the disease in areas where the disease is not known to occur. Strict quarantine regulations have been imposed on the import of bunted grain. In 1996, the Governments of Turkey, Morocco, and Poland detained some wheat export consignments from India. A cargo worth US $5 million was not allowed to be unloaded in Poland due to Karnal bunt infection causing potential losses to export market of India (Kumar et al. 2015).

Quarantine also needs to be set up at the domestic level to restrict the movement of diseased material from one state to another. Pearl millet seeds carry the downy mildew pathogen (*Sclerospora graminicola*) in the form of oospores sticking to the seed surface. Sclerotia of ergot occur as concomitant contaminant of seed during harvest. These can be detected and excluded at the plant quarantine level by proper cleaning of seeds before sowing.

25.2.1.2 Enforcement of Seed Certification Standards

Use of certification programs for the production of disease-free seeds/planting material for vegetatively propagated plants is a fundamental and practical strategy for excluding pathogens. These certification programs utilize techniques like crop-wise isolation distances, various field inspections at different crop growth stages, and removal of infected plants for production of disease-free seed and planting material. Use of tissue culture and micropropagation techniques promotes the increase in pathogen-free planting stocks which can be grown in greenhouses to create a barrier for the disease incitants and their spreading agents. Exclusion can be

simply achieved by cleaning of agricultural equipments such as tractor, cultivator, seed drill, thresher, combine, etc. to remove contaminated debris, soil particles, and infected seeds that can have range of pathogens such as *Fusarium*, *Verticillium*, nematodes, and other soil-borne or seed-borne microorganisms and restrict their entry in disease-free areas (Maloy 2005).

Seed health testing is an integral component of seed certification programs and is being used in integrated disease management. Seed health certification is a legalized scientific exercise to secure, maintain, multiply, and make available quality seed to the consumers. It is the privilege of the government to lay down the procedures of certification and fix a minimum permissible limit for a particular disease. This limit may vary from zero to any number depending upon the virulence of the pathogen and the susceptibility of host. It involves testing of seeds before sowing and after harvest and crop inspection for compliance of standards including isolation and freedom from weeds and diseases (Hewett 1979). The International Seed Testing Association (ISTA) is engaged in the development of standardized seed testing procedures that are available in the form of international rules for seed testing (ISTA 2018). In India, the Central Seed Certification Board (CSCB) is responsible for framing the rules and procedures for seed certification including seed health. CSCB has fixed the permissible limit for few seed-borne diseases on the basis of field inspections or seed evaluations (Tables 25.2 and 25.3). However, under the provisions of Seed Act 1966, labeling is compulsory, but certification is voluntary.

According to Indian minimum seed certification standards, loose smut is an objectionable designated disease at the field level. There are no prescribed seed standards fixed for seed lots for loose smut infection. However, the Central Seed Certification Board has permitted a maximum of 0.10% and 0.50% infected plants in the foundation and certified seed production plots, respectively. An isolation distance of 150m is also recommended from the field of wheat infected by the disease. A minimum of two inspections should be made between the ear emergence and harvesting of the seed crop (Tunwar and Singh 1988). In the UK, the standards for loose smut are 0.2, 0.5, and 0.5% for basic seed, first-generation certified seed, and second-generation certified seed, respectively (Rennie et al. 1983). In the USA, most breeder, foundation, registered, or certified seed classes specify a zero or near-zero tolerance for the disease (Mathre 1982). As a result of seed certification and seed treatment protocols, the incidence of loose smut disease in the seed production program for wheat has reduced tremendously over the years.

25.2.2 Eradication

The principle of eradication is based on elimination of a pathogen after its introduction into an area but prior to its well establishment or widespread dispersal. This is effective in case of individual plants, seed lots, and small areas but generally not applicable for large areas (Maloy 2005). The methods aimed (a) to avoid contact of the host with the pathogen or (b) to avoid coinciding of the susceptible stage of the plant with the favorable conditions for the inoculum buildup. These methods help to

Table 25.2 Certification standards for foundation seed and certified seed based on maximum percent seed infection

Crop species	Disease	Causal organism	Certification standards	
			Foundation seed	Certified seed
Pearl millet	Ergot	*Claviceps fusiformis*	0.02	0.04
Paddy	Bunt	*Neovossia horrida*	0.10	0.50
Sorghum	Ergot	*Sphacelia sorghi*	0.02	0.04
Wheat	Ear cockle	*Anguina tritici*	None	None
	Karnal bunt	*Neovossia indica*	0.05	0.25
	Tundu	*Corynebacterium michiganense* pv. *tritici* and *Anguina tritici*	None	None
Triticale	Karnal bunt	*Neovossia indica*	0.05	0.25
	Ergot	*Claviceps purpurea*	0.02	0.04
Potato	Late blight	*Phytophthora infestans*	1.0	1.0
	Dry rot	*Fusarium caeruleum*	1.0	1.0
	Charcoal rot	*Macrophomina phaseolina*	1.0	1.0
	Wet rot	*Sclerotium rolfsii*	None	None
	Common scab	*Streptomyces scabies*	3.0	5.0
	Black scurf	*Rhizoctonia solani*	3.0	5.0
	Total diseases	–	5.0	5.0
Onion (bulblets)	Basal rot	*Fusarium oxysporum* f. sp. *cepae*	None	None
	Soft rot	*Erwinia carotovora*	None	None
	Brown rot	*Pseudomonas aeruginosa*	None	None
Sweet potato	Storage rot	Complex etiology	None	None
	Black rot	*Ceratostomella fimbriata*	None	None
	Scurf	*Monilochaetes infuscans*	None	None
	Wilt	*Fusarium oxysporum* f. sp. *batatas*	None	None
	Internal cork	Virus	None	None

reduce the source of infection in an established disease primarily by breaking the disease cycle. To do so, it is essential to know the life cycle of pathogen, its mode of perennation, host range, and growth habit for devising control strategy for a disease.

25.2.2.1 Identification of Disease-Free Areas for Healthy Seed Production

Selection of suitable area or field for cultivation of crops for better yields and also for protection against diseases is essential. Healthy seed production is advisable in areas and seasons where disease does not appear. The hybrid seed of pearl millet free from ergot disease is produced in Gujarat and distributed in entire north India. Many fungal and bacterial diseases are more severe in wet areas than in dry areas, e.g., ergot and smut diseases in pearl millet. It is also advisable not to use the same

Table 25.3 Certification standards for foundation seed and certified seed plots based on percent maximum disease incidence in field (field standards)

Crop	Disease	Causal organism	Certification standards		Stage of inspection
			Foundation seed	Certified seed	
Barley	Loose smut	*Ustilago nuda*	0.10	0.50	Between ear emergence to harvest
Wheat	Loose smut	*U. tritici*	0.10	0.50	- do -
Triticale	Loose smut	*U. tritici*	0.10	0.50	- do -
	Ergot	*Claviceps purpurea*	0.02	0.04	At maturity prior to harvest
Sorghum and forage sorghum	Kernel smut or grain smut	*Sphacelotheca sorghi*	0.05	0.10	- do -
	Head smut	*Sphacelotheca reiliana*	0.05	0.10	- do -
Pearl millet	Downy mildew/green ear	*Sclerospora graminicola*	0.05	0.10	Before flowering stage to maturity
	Ergot	*Claviceps fusiformis*	0.02	0.04	At maturity prior to harvest
	Grain smut	*Tolyposporium penicillariae*	0.05	0.10	- do -
Cowpea	Ashy stem blight	*Macrophomina phaseolina*	0.10	0.20	At flowering and fruit stage
	Anthracnose	*Colletotrichum lindemuthianum*	0.10	0.20	- do -
	Ascochyta blight	*Ascochyta* spp. (for hill areas only)	0.10	0.20	- do -
French bean	Bacterial blight	*Xanthomonas* spp.	0.10	0.20	- do -
	Anthracnose	*Colletotrichum lindemuthianum*	0.10	0.20	- do -
	Ascochyta blight	*Ascochyta phaseolorum* (for hill areas only)	0.10	0.20	- do -
	Bean mosaic	*Bean common mosaic virus*	0.10	0.20	- do -
Sesame	Leaf spot	*Cercospora sesami*	0.50	1.0	At and after flowering
Sunflower	Downy mildew	*Plasmopara halstedii*	0.05	0.50	At stage of 6–7 pairs of leaves to maturity prior to harvest
Jute	Jute chlorosis	Virus	1.0	2.0	At maturity prior to harvest

(continued)

Table 25.3 (continued)

Crop	Disease	Causal organism	Certification standards		Stage of inspection
			Foundation seed	Certified seed	
Guar	Bacterial blight	*Xanthomonas campestris* pv. *cyamopsis*	0.10	0.20	At flowering and fruit stage
	Anthracnose	*Colletotrichum lindemuthianum*	0.10	0.20	- do -
	Ascochyta blight	*Ascochyta* spp. (for hill areas only)	0.10	0.20	- do -
Oat	Loose smut	*Ustilago avenae*	0.10	0.50	At ear emergence to harvest
Colocasia	Phytophthora blight	*Phytophthora colocasiae*	None	None	At all three stages of inspection
Muskmelon	Cucumber mosaic virus	*Cucumber mosaic virus*	0.1	0.2	- do -
Summer squash	Cucumber mosaic virus	*Cucumber mosaic virus*	0.1	0.5	At mature fruit stage prior to harvesting
	Watermelon mosaic virus	*Watermelon mosaic virus*	0.1	0.5	-do-
Brinjal	Phomopsis blight	*Phomopsis vexans*	0.1	0.5	- do -
Capsicum	Leaf blight	*Alternaria solani*	0.1	0.5	- do -
	Anthracnose	*Colletotrichum capsici*	0.1	0.5	- do -
Radish	Black leg	*Leptosphaeria maculans*	0.1	0.5	At flowering stage
	Black rot	*Xanthomonas campestris* pv. *campestris*	0.1	0.5	- do -
Tomato	Early blight	*Alternaria solani*	0.1	0.5	At mature fruit stage prior to harvest
	Leaf spot	*Stemphylium solani*	0.1	0.5	- do -
	Mosaic	*Tobacco mosaic virus*	0.1	0.5	- do -
Celery	Leaf blight	*Septoria apiicola*	0.1	0.5	- do -
	Root rot	*Phoma apiicola*	0.1	0.5	- do -
Lettuce	Lettuce mosaic virus	*Lettuce mosaic virus*	0.1	0.5	At flowering stage
Parsley celeriac	Leaf spot	*Septoria petroselini*	0.1	0.5	At maturity prior to flowering

(continued)

Table 25.3 (continued)

Crop	Disease	Causal organism	Certification standards		Stage of inspection
			Foundation seed	Certified seed	
Cabbage	Black leg	*Leptosphaeria maculans*	0.1	0.5	- do -
Cauliflower					
Knol Khol	Soft rot	*Erwinia carotovora*	0.1	0.5	- do -
Broccoli	Black rot	*Xanthomonas campestris* pv. *campestris*	0.1	0.5	- do -
Turnip	Black rot	*Xanthomonas campestris* pv. *campestris*	0.1	0.5	At flowering stage
Sweet potato	Black rot	*Ceratostomella fimbriata*	None	None	Inspection at plant bed stage
	Wilt	*Fusarium oxysporum* f. sp. *batatas*	None	None	- do -
	Scurf	*Monilochaetes infuscans*	None	None	After transplanting
	Mosaic	Virus	0.05	0.10	- do -
Chinese cabbage (heading and non-heading)	Black leg	*Leptosphaeria maculans*	0.1	0.5	- do -
	Black rot	*Xanthomonas campestris* pv. *campestris*	0.1	0.5	- do -

field for cultivation of crop year after year. Selection of same field may aggravate, for example, wilt of arhar, late blight of potato, stem gall of coriander, green ear disease, smut, or ergot diseases of pearl millet. The drainage in the field is also important since low-lying, waterlogged conditions favor diseases as red rot of sugarcane, downy mildew of pearl millet, damping off, and root rots in various agricultural crops.

Phytopathogens present in the soil pose a serious threat in vegetable cultivation in most countries particularly seed production of vegetable crops. Non-infested soil and safer areas are crucial for cultivation of different crops. Plowing hampers the disease-causing organisms present in soil and carried over crop residues. The length of fallow period is negatively correlated with the pathogenic population. The inoculum load of soil-borne pathogens can be lowered by integration of various cultural practices along with fumigation of soil and use of biological agents. Compaction of soil needs to be avoided as it hinders root growth and favors diseases caused by retention of excess soil moisture. Raised beds should be preferred for better drainage. Soil should be tested for nutrient status and nematode incidence before sowing a crop. The historical knowledge on outbreak of soil-borne disease is helpful for predicting the possible problems in the future.

Plant to plant spacing, row to row distance, time and amount of irrigation, quantity and quality of fertilizers or organic manures, time and method of sowing/planting, mixed cropping method, depth of sowing, etc. are some of the cultural practices which influence the incidence and severity of certain diseases. Overcrowding may result in damping off of seedlings. The incidence of bunt and flag smut in wheat is higher in deep sown crops. Deep plowing of soils that contain diseased potato tubers can effectively reduce the amount of inoculum of *Phytophthora infestans*. Sowing of trap crops stimulates the dormant pathogen, and thus the actual host gets protected from pathogen's attack. Mixed cultivation of cotton and moth reduces the incidence of root rot in cotton caused by *Rhizoctonia.* Balanced application of fertilizers particularly nitrogenous fertilizers also reduces severity of diseases.

25.2.2.2 Time of Sowing

The time of sowing also influences the incidence of diseases. Pathogens are able to infect susceptible plants only under certain environmental conditions, e.g., downy mildews require prolonged high moisture for infection. Root rot caused by *Rhizoctonia* is severe if gram is sown immediately after rains. The early maturing wheat and pea varieties also escape damage due to *Puccinia graminis tritici* and *Erysiphe polygoni*, respectively. Use of pathogen-free seed material is the most effective method for disease-free seed production. In India, seed potatoes are invariably imported in the southern states from Shimla hills for the control of viral and bacterial diseases. In the USA, seed growing areas have been shifted to dry pacific regions for crops such as cabbage, turnip, beans, and peas for obtaining disease-free seed and indirectly controlling diseases as black leg and black rot of cabbage and turnip, etc. Change in sowing or planting dates is helpful to minimize disease occurrence.

25.2.2.3 Cultural Practices

Cultural practices contribute significantly in the control of many plant diseases. The aim of cultural practices is to provide favorable environmental conditions for crop development resulting in good plant health and to restrict the growth of phytopathogens that minimizes the disease incidence. Modifications in several cultural practices are important for management of any crop disease. Some common practices are selection of safe areas and crop seasons, proper tillage to destroy diseased plant residues, cultivation of non-host crops, selection of disease-free seed/planting materials, proper direction of crop to facilitate proper sun and air exposure, adequate irrigation, and nutrition management of crop spacings and population which improves root growth, avoids injury to the plants, reduces foci of inoculum, and facilitates proper aeration in the fields.

25.2.2.4 Irrigation Management

Survival of the pathogen and disease development can be restricted using irrigation water judiciously thus creating an unfavorable environment for the pathogen. Low areas should be avoided, and measuring devices like tensiometers can be helpful in irrigation management and thereby utilizing resources in best manner and also in

disease management (Montesano et al. 2015). High soil moisture enhances seed rots and decay in field and promotes the population of soil-borne phytopathogens, like *Phytophthora* spp. and *Pythium* spp. Water stagnation in the field adversely affects roots due to non-availability of oxygen which creates favorable conditions for development of infection by soil-borne phytopathogens. An excellent example of cultural management of plant diseases is the root and stem rots, wherein the incidence of these diseases can be minimized using raised plant beds which ensures good drainage of water.

25.2.2.5 Soil and Fertilizer Management

Various diseases get affected due to the plant nutrition and soil pH. Occurrence of *Fusarium* wilt on tomato can be minimized by application of fertilizers containing high amount of nitrate nitrogen (NO3) than ammoniacal nitrogen (NH4) (Borrero et al. 2012). Application of lime is a good management strategy for reducing the severity of *Fusarium* wilt and *Botrytis* gray mold diseases as it increases the soil pH. Severity of bacterial wilt disease can also be reduced with optimal calcium nutrition and higher soil pH in the field. Blossom end rot disease can be reduced by addition of adequate calcium which also promotes overall healthy growth. Judicious use of nitrogen provides less dense plant canopies thereby ensuring proper air circulation in the crop field, leading to unfavorable condition for development of many diseases (Dordas 2008).

25.2.2.6 Roguing of Infected Plants at Proper Time

The infected plants or plant parts should be carefully removed from the fields and destructed at an early stage to remove foci of infection and restrict spread of the pathogen. Roguing of smutted ear heads in diseases as loose smut of wheat, loose smut and covered smut of barley, sorghum, or maize, and ergot of pearl millet and whole plant in case of wilt of arhar, yellow vein mosaic in okra, etc., as early as possible, is essential to prevent the spread of infection. The infected ear heads are carefully covered with long paper/polythene bags, and the ears are plucked with the scissors causing minimum dislodging of spores and destroyed by burning or burying deep in a pit. To control bacterial and viral diseases, uprooting and destroying the diseased plants is the best strategy.

25.2.2.7 Eradication of Alternate or Collateral Hosts

Many pathogens require two hosts for completing their life cycles. The destruction of one wild host breaks the life cycle of the pathogen. Many plant pathogens survive on weeds which serve as alternate/collateral hosts, e.g., *Yellow vein mosaic virus* of okra survives on *Hibiscus tetraphyllus*. Destruction of this wild host by proper weeding can thus contain the disease. Barberry eradication is actually a form of protection and not elimination of pathogen for minimizing stem rust of wheat caused by *Puccinia graminis*. The strategy is based on breaking the disease cycles by eradication of alternate host and preventing infection of the economically more valuable host. However, the urediospores of the stem rust pathogen are capable of

causing infection from wheat to wheat in many areas, but eradication of barberry, the aecial host, may minimize the development of pathogenic races of the stem rust.

25.2.2.8 Crop Rotation

Some seed-borne diseases may also be soil-borne in nature, e.g., Bakanae disease of rice (Gupta et al. 2015). Crop rotation is a very important practice, especially for the reduction of soil-borne inoculum of the pathogens. Continuous cultivation of the same crop in the same field (monocropping) leads to buildup of inoculum levels of the pathogen in the soil thus making them sick. Crop rotation is a commonly used management practice to minimize the pathogenic load usually of soil-borne or seed-borne pathogens which also have capability to survive in soils, in a cropping area. Proper crop rotation helps to control many soil-borne diseases as wheat mosaic; wilt of pigeon pea, linseed, pea, and gram; red rot and wilt of sugarcane; ergot and smut of pearl millet; and root rot of vegetable crops. Short crop rotations with non-host crops of 1 and 2 years is helpful in the management of soil-borne diseases like take-all disease of wheat caused by *Gaeumannomyces graminis* and soybean cyst nematode, *Heterodera glycines*, respectively. Crop rotation also helps to replenish the soils with essential nutrients. A minimum of 3-year crop rotation with a non-host crop is advisable for many soil-borne diseases, which significantly causes reduction in populations of the pathogen. This strategy is useful for management of diseases like *Phytophthora* blight of pepper and *Fusarium* wilt of watermelon, though they may need longer crop rotation periods of up to 5–7 years. If possible, land cultivated with alternate and reservoir hosts in the previous cropping seasons should not be selected. The disease inoculum and insect vector sources should be located as far away as possible from the vegetable fields.

25.2.2.9 Field Sanitation

Field sanitation strategies are essential for management of various infectious plant diseases by excluding, minimizing, or elimination of the pathogen. Pathogens survive through dormant structures in the soils. Hence, it is essential to collect and destroy all these structures and diseased stubbles. The soils infested with phytopathogens should not be moved from one area to another, for example, the sclerotia of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* present in the infested soils are primarily carried from one place to another. If possible, allow fallow period between two successive crops especially during hot summers when the land is exposed to hot sun after plowing. Solarization also helps to solve majority of problems. The soil bed should be made wet and covered with black polyurethane sheets so that no moisture escapes through the sides. The bed should remain so for a week wherein the soil gets sterilized and free of soil-borne diseases, nematodes, and other pests. Use of disease-free seeds and planting stocks is crucial for disease management.

Crop rotation and destruction of volunteer plants challenge the survival of pathogens from one season to another. Postharvest diseases can be minimized by safe handling of crop/product which reduces wounds during harvesting and packing. Destruction of weeds which harbor several pathogens or their insect vectors also helps in their eradication. An effective method of eradicating the overwintering

inoculum of the late blight pathogen is by eliminating potato cull piles. Viral diseases can also be effectively managed through weed control as they are reservoir for many important vegetable viruses. Eradication of weeds also reduces the primary inoculum. The reduction in the populations of weed and primary inoculum of soilborne pathogens can also be achieved through non-host cover crops.

Fumigation of soil is a commonly used technology for eradication of phytopathogens. This technique is used to kill the target pathogens by penetration of gas-forming chemicals like carbon disulfide, methyl bromide, or chloropicrin into the soil. The field is sealed with a plastic film after volatile fumigants like methyl bromide are injected into soil. The soil can be simply compacted to form a seal after some water-soluble fumigants like metam-sodium are injected into the soil. However, this technique is less reliable for disease management as it results in undesirable side effects like killing of beneficial organisms, contamination of groundwater, and toxicity of the fumigant chemicals. Solarization can also be used for field sanitation to some degree, depending on crops and other factors.

Polyethylene mulch provides a physical barrier between soil and the aerial plant parts. This approach is used for fruit rot control in the field for vegetables. Metalized mulches that are highly UV-reflective repel some insect vectors that transmit viruses and are beneficial when incidence of insect vectors of some viral diseases is high during the year. Metalized mulches have been found to effectively reduce incidence of TSW and associated thrip populations on tomatoes, but these cannot be used under low soil temperatures especially during winter season.

Agricultural operations like thinning, pruning, or tying of plants help in spread of the pathogens like bacteria. These operations may cause wounds on the host plants which facilitate easy entry of some pathogens. Hence plants should be handled carefully when dry to avoid injury. Optimum plant populations in the field with proper spacings have an open canopy resulting in low moisture which hampers growth of most pathogens. Incorporation of composted organic amendments in the soil increases aeration and drying of the soil. Additionally, incorporation of organic amendments promotes populations of beneficial microbes and thereby reduces pathogenic populations (Kumar 2010; Kumar et al. 2010, 2017b). The pathogen inoculum can be reduced by removing plant debris after harvest. Workers should clean their hands and agricultural implements time to time using a disinfectant, like isopropyl alcohol especially during crop pruning and harvesting. If possible, staking and tying of plants should be done to improve air circulation in the crop canopy.

In the USA, the golden nematode is eradicated by removing infested soil, soil fumigation, and discarding nematode-affected potato fields (Maloy 2005). Pathogens can be effectively eradicated by burning which is often required by law in the USA to remove elm trees affected by Dutch elm disease, citrus trees infected by citrus canker, or bean fields infected by halo blight bacteria, *Pseudomonas syringae* pv. *phaseolicola* (Maloy 2005). On a modest scale eradication can also be accomplished by removing apple or pear branches infected by the fire blight bacterium (*Erwinia amylovora*) or pruning of white pine branches to remove blister rust cankers caused by *Cronartium ribicola*, or the diseased flower bulbs, corms, or rhizomes can be sorted and removed. Other examples of eradication of the pathogen are through

treatment of cereal seeds with hot water to kill smut mycelium and heat treatment to eliminate viruses from fruit tree budwood for grafting. *Verticillium* microsclerotia in mint stems can be effectively destroyed by propane flaming, and tuber-borne infection of *Phytophthora infestans*, incitant of potato late blight disease, can be stopped, if potato stems are flamed prior to harvesting. However, burning of agricultural fields is a debatable issue because the smoke produced due to burning is hazardous for human health, safety, and the environment (Maloy 2005).

Eradication of a pathogen as cited in above examples can be conflicting as they can be placed under protection also, since complete eradication of pathogens is rarely accomplished especially from large regions.

25.2.3 Protection

The principle of protection is to create barriers between the phytopathogen and the host plant or the susceptible part of the host plant. Protection is usually accomplished as a chemical barrier, e.g., a fungicide, bactericide, or nematicide, or a physical, spatial, or temporal barrier usually involving some cultural practice that alters the environment that helps plants to escape disease infection or minimize disease severity. Specific measures are adopted assuming that phytopathogens are present which will cause infection in the absence of suitable protective techniques. For example, date of sowing is manipulated to protect the crops from blights, leaf spots, downy mildew diseases, etc. The crops can be protected against the diseases either by application of chemicals on the seeds/propagative material or by foliar applications in the field. Application of chemicals to seed before sowing helps in the elimination of seed-borne pathogens that cause seed rots, seedling blights, bunts, or smuts and also protects the germinating seed from the attack of soil-borne phytopathogens. Seed treatment is a simple yet the cheapest and safest method of controlling different pests. Seed is treated to promote good seedling establishment, to minimize yield loss, to maintain and improve their quality, and to avoid the spread of harmful organisms. Different physical, chemical, or biological seed treatment methods have been proposed from time to time for control of various diseases. The inoculum present as external contaminant, e.g., spores, mycelium, ergot, or sclerotia, can be removed from the seed lot by physical methods as sieving, steeping in water, or brine solution. Heat therapy can be used for eradication of internal infections in the seed lots.

25.2.3.1 Hot Water Treatment

Hot water treatment has proved to be effective in controlling the diseases for a long time. Soaking seed for 5–6 h in water at 20–30 °C and then dipping it for 2 min in hot water at a temperature of 50 °C followed by drying the seed kill the dormant mycelium of *Ustilago segetum tritici* causing loose smut of wheat (Jensen 1888). Lambat et al. (1974) reported eradication of fungus *Phoma betae* in sugar beet seed by hot water treatment at 50 °C for 30 min. The treatment has also been found effective in the control of seed-borne bacterial diseases as in rice (*Xanthomonas oryzae*),

Table 25.4 Control of seed-borne pathogens by hot water treatment of seed (Chaube and Singh 1990)

Host	Disease	Pathogen	Treatment
Brassica spp.	Black rot	*Xanthomonas campestris* pv. *campestris*	50 °C for 20 or 30 min
Cluster bean, guar (*Cyamopsis tetragonoloba*)	Blight	*X. campestris* pv. *cyamopsidis*	50 °C for 10 min
Cucumber (*Cucumis sativus*)	Seeding blight	*Pseudomonas syringae* pv. *lachrymans*	50 °C and 75% RH for 3 days
Groundnut (*Arachis hypogaea*)	Testa nematode	*Aphelenchoides arachidis*	60 °C for 5 min after soaking for 15 min in cool water
Lettuce (*Lactuca sativa*)	Leaf spot	*X. campestris* pv. *vitians*	70 °C for 1–4 days
Pearl millet (*Pennisetum typhoides*)	Downy mildew	*Sclerospora graminicola*	55 °C for 10 min
Potato (*Solanum tuberosum*)	Potato phyllody	*Phytoplasma*	50 °C for 10 min
Rice (*Oryza sativa*)	Udbatta	*Ephelis oryzae*	54 °C for 10 min
	White tip	*Aphelenchoides besseyi*	51–53 °C for 15 min after dipping for 1 day in cool water
Safflower	Leaf spots	*Alternaria* spp.	50 °C for 30 min
Teasel (*Dipsacus* spp.)	Stem nematode	*Ditylenchus dipsaci*	50 °C for 1 h or 48.8 °C for 2 h
Tobacco (*Nicotiana tabacum*)	Hollow stalk	*Erwinia carotovora* pv. *carotovora*	50 °C for 12 min
Tomato (*Lycopersicon esculentum*)	Black speak	*Pseudomonas syringae* pv. *tomato*	52 °C for 1 h

cotton (*Xanthomonas axonopodis* pv. *malvacearum*), cauliflower (*Xanthomonas campestris* pv. *campestris*) seeds, etc. The efficacy of hot water treatments at 40 °C, 50 °C, and 55 °C for 10–30 min, on seeds of five vegetable crops, viz., carrot, cabbage, celery, parsley, and lamb's lettuce, was tested. The efficacy of hot water treatments was obtained above 95% against *Alternaria* species (*A. dauci*, *A. radicina*, *A. alternata*, *A. brassicicola*). Against *Xanthomonas campestris* on carrot and cabbage, hot water seed treatment laboratory trials exhibited good effects at 50 °C for 30 min (Nega et al. 2003). However, hot water treatment is laborious and demands expertise. A range of temperatures with various exposure time have been tested for hot water treatment in various crops (Table 25.4), which resulted in successful control of different phytopathogens (Chaube and Singh 1990). The thermal death point of the pathogens and the embryo are very near to each other, and therefore extensive care is needed to exactly control the temperature for which controlled water baths are essential and hence the method has not become popular.

25.2.3.2 Solar Heat Treatment

To eliminate *Ustilago tritici*, the fungal incitant of loose smut disease of wheat, a simplest technique has been developed in India. This method has advantage that it eliminates the risk of killing of the embryo during hot water treatment used previously to eradicate *Ustilago tritici* from wheat seeds. Since the thermal death point of the fungus and the embryo are very close, extensive care is required to be taken to avoid killing of the embryo in hot water treatment.

In the plains of North India, the summer temperatures are very high (42–44 °C) during the months of May and June. Luthra and Sattar (1934) suggested to soak the seed in water for 4 h in the forenoon on a bright summer day followed by drying for 4 h in the sun. Bedi (1957) found that drying even for 1 h is sufficient to kill the intraseminal mycelium of *Ustilago segetum tritici* under Punjab conditions, and they further suggested to store the seed carefully after drying it completely.

25.2.3.3 Anaerobic Treatment

The seed is soaked in water at 15–20 °C for 2–4 h. This moist seed is then kept in airtight containers for 65–70 h and thereafter dried (Zemanek and Bartos 1964). In this method the seed is deprived of air for up to a week.

25.2.3.4 Hot Air Treatment

The dry heat seed treatment is quite effective and less injurious to seed. Hot air treatment at 54 °C for 8 h has been reported to be capable of managing ratoon stunting disease (RSD) in sugarcane without affecting the germination of sugarcane buds (Lauden 1953). Similarly, grassy shoot disease of sugarcane has been controlled by hot air at 54 °C for 8 h (Singh 1968). Singh (1973) reported successful control of red rot disease in different sugarcane varieties by hot air treatment at 54 °C for 8 h. *Pseudomonas syringae* pv. *phaseolicola* in beans could be reduced, if seeds were exposed to 50 °C for 3 days, without adversely affecting seed germination (Tamiethi and Garabaldi 1984). The seed-borne bacterial pathogen *X. campestris* pv. *translucens* is responsible for bacterial black chaff and leaf streak of cereal grains. Dry heat treatment at 71 °C, 75 °C, or 84 °C for 11 days successfully eliminated *X. campestris* pv. *translucens*, from heavily infested barley seed without a considerable reduction in seed germination, whereas seed exposure to 72 °C for 4 day was capable to eliminate this pathogen from moderately infested barley seed (Fourest et al. 1990). Likewise, the incidence of *P. syringae* pv. *pisi* in pea was reduced when seeds were exposed to 56 °C for a day without affecting germination (Grondeau et al. 1992). Hot air therapy has been used for the control of certain viruses as in peach and tomatoes. Hot air treatments of carrot seed have the best remedial effects against *Alternaria dauci* and *A. radicina*, which were as effective as chemical treatments (Koch et al. 2010).

25.2.3.5 Chemical Treatments

Use of agrochemicals in general and pesticides in particular is widespread in agriculture to limit various pests of crop plants including diseases. Fungicides and bactericides are important in many disease management programs. It is essential to

integrate chemicals with all other appropriate strategies discussed here. Foliar application of chemicals protects the plants against the pathogens which attack the growing plants. These sprays can be preventive or curative. Depending on the type of action, these chemicals can be surface protectants or systemic in nature. The protectant pesticides are prophylactic in their action and form a protective barrier on the surface of the seed/plant system that discourages the growth of the pathogen. The systemic pesticides penetrate the plant tissues and kill the pathogen already established in the tissues. Fungicides have been in use for more than a 100 years against various diseases, and development of novel formulations for the fungicides is a continuous process. The first widely used fungicide was Bordeaux mixture, which is a copper sulfate fungicide, and is being used presently in various forms. The initial groups of inorganic fungicides were derived from simple elements like sulfur or metallic compounds of copper or mercury. Organic fungicides like thiram, captan, and bisdithiocarbamates developed in the early to mid-1900s are broad-spectrum, contact, or protectant fungicides that are effective against various fungal pathogens. The systemic fungicides were developed in the 1960s, though most of them are not truly systemic but are usually translaminar having limited mobility. These often offer certain post-infection benefits. Some systemic fungicides move only in the upward direction in the vascular system of the plant, but presently fungicides moving in both upward and downward directions are available, e.g., fosetyl-Al, which is a truly systemic fungicide. Strobilurins are a recent group of systemic fungicides. Some fungicides are effective against a large number of diseases, but some of them are effective against specific groups of diseases like downy mildews, rusts, smuts, or powdery mildews.

These recent fungicides have narrow range against target microbes because of their site-specific mode of action which is controlled by one or few genes and hence develop resistance in the phytopathogens quickly. To resolve this problem of fungicide resistance in the target organisms, various management strategies have been developed. It is essential to use chemicals at recommended doses and at proper time. Also, the most effective molecule should be selected along with proper equipment and appropriate technique for its application. Correct diagnosis of the disease is always the basis for successful management of the diseases. The labels on pesticides should always be read and the instructions should be followed carefully. Knowledge on the physical mode of action of a fungicide helps to determine its proper application timing. These physical modes of action of fungicides can be grouped into four categories, i.e., protective, post-infection, before, and after the appearance of disease symptoms. Protectant fungicides include molecules used as foliar spray such as copper compounds and mancozeb, which are effective when applied on the leaf (or plant) surface before the infection. Systemic fungicides which are therapeutic in nature are either true systemic or translaminar or meso-systemic and are active inside of the leaf as they can penetrate the plant differentially through the cuticle. Systemic fungicides are able to check the already initiated infection and prevent further development of disease. Rotation of fungicides is recommended to tackle problem of fungicide resistance for effective management strategies. This can be achieved through use of mixtures containing single and multi-site fungicides,

rotating application of molecules with different modes of action, and need-based use of fungicides instead of calendar based at the recommended dose. Application of fungicides can be done by any of various means as ground sprayers or through airplanes on large areas or through irrigation systems, but their applications must be done properly for good effectivity. The other most important factors of the fungicides are their efficacy, cost, safety, ease, and timing of application. Timing of application of fungicides is very critical since the chemical is ineffective and gets wasted, if applied early or too late. Application of fungicide is also important as the finer sprays are distributed more evenly on the plant surface, though very small droplets form a mist that are easily carried away by the wind. However, a new management strategy to trigger the plant's defense reaction without direct interference with the pathogen has been developed to manage plant diseases chemically. Acibenzolar-S-methyl is a registered chemical in this category and now being used commercially for the control of bacterial spot and bacterial speck on tomatoes (Graves and Alexander 2002). Fumigants can be used to manage soil-borne pathogens. However, before applying, it is important to review the disease history of the area, when choosing fumigant materials.

Captan and thiram are extensively used as seed dressing fungicides against damping off of beans, chillies, pea, tomato, seedling rot of groundnut, seed rots and blights of maize, etc. The use of systemic fungicides like carboxin, carbendazim, propiconazole, raxil, etc. has become quite popular due to their effectiveness against a number of devastating diseases like loose smut of wheat, blast of paddy, downy mildew in bajra, seedling rot of sugar beet, head smut of maize, etc. both as seed and foliar treatments. Antibiotics like streptomycin and tetracycline are most effective against phytobacteria. The incidence and secondary infections of viral diseases can be reduced by effective management of their insect vectors as whiteflies, thrips, and aphids.

The chemical pesticides, especially the fungicides, are of high value in plant protection, but the problem of residue buildup in the produce, development of resistance in pathogens, adverse effect on non-target species, and environmental pollution make it impertinent and there is a need to look for other alternatives. Nevertheless, use of chemicals for the protection of plants against diseases can be integrated with other strategies for disease management that do not have associated health risks.

25.2.3.6 Biological Control

Biological control is a key component in the concept of integrated disease management. Biological control has emerged as a trend in the field of plant disease management in recent few decades using one living organism to control another. Bio-products derived from plants and biopesticides or popularly called as biocontrol agents have shown potential to check various plant diseases. Biocontrol agents mean the microbes capable of reducing the population of target pests (e.g., phytopathogenic microorganism and/or insect pest) growing in association with them. Generally, biological control agents (bca) are capable to arrest the life process of phytopathogens. Biocontrol agents include different types of microorganisms, e.g., fungi, bacteria,

nematodes, viruses, etc. Their easy availability, rapid biodegradation, low toxicity, self-perpetuation, compatibility with other products, ecological and environmental safety, and other properties make them more reliable and reduce the usage and dependence on chemical pesticides. They control the pathogens through antagonistic activity of microorganisms, which is achieved by parasitism, predation, competition, or commensalisms (Mukherji 1983). However, till date the biological control agents are not registered in great numbers as required, their efficacy is not guaranteed in each and every cropping system, and the use of biocontrol agents is limited to high value crops grown usually under protected cultivation which requires intensive management. Two most popular examples where biological control has been effective are prevention of infection of adjacent trees by *Heterobasidion annosum*, a wood-decaying fungus by inoculating tree stumps with *Peniophora gigantea* fungus, and prevention of infection by the crown gall bacterium (*Agrobacterium tumefaciens*) by using *Agrobacterium radiobacter*, a nonpathogenic and non-tumor-forming bacterium, to fruit trees before planting (Maloy 2005).

The important fungal genera which are known as potential biocontrol agents against different phytopathogens include *Trichoderma*, *Gliocladium*, *Aspergillus*, *Penicillium Neurospora*, *Chaetomium*, *Dactylella*, *Arthrobotrys*, *Catenaria*, *Paecilomyces*, *Glomus*, etc. Serval bacterial species have been utilized as biocontrol agents. Important bacterial genera include *Agrobacterium*, *Actioplanes*, *Alcaligenes*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Pasteuria*, *Rhizobium*, *Serratia*, *Streptomyces*, etc. Some of the biocontrol agents that are available commercially are the fungi viz., *Trichoderma asperellum*, *T. harzianum*, and *Gliocladium virens*; an actinomycete viz., *streptomyces griseoviridis*; and the bacteria viz., *Bacillus subtilis* and *Pseudomonas fluorescens*. Bacterial spot on tomato has been effectively managed using bacteriophages (phages) which are viruses that infect bacteria exclusively.

T. viride and *T. harzianum* have been reported to control seed rot and damping off diseases in chickpea, incited by a fungal pathogen *Pythium ultimum* (Shahriary et al. 1996). In maize and sorghum seed rot and early stem rot diseases are caused by *F. moniliforme*, *Rhizoctonia solani*, and *Pythium ultimum.* These seed-associated diseases can be controlled by using *T. harzianum* (D'Ercole et al. 1988). The potential of *T. viride* and *C. globosum* was tested against seed-borne fungal incitants of pigeon pea, and successful control was obtained (Pradeep et al. 2000). *P. fluorescens*, capable in significant management of several phytopathogenic fungi, is an extensively explored bacterium. This bioagent reduces the seed-borne infection in radish (Leeman et al. 1995) and sorghum (Raju et al. 1999). The use of bioagents may result in increased percent germination due to the reduction in fungal incidence on seeds. Four bioagents, viz., *T. viride*, *T. harzianum*, *C. globosum*, and *P. fluorescens*, significantly increased the germination of five different cultivars of sorghum seeds infected by *F. moniliforme* (Raju et al. 1999). Significant control of stem gall disease of coriander and significant increase in seed yield of this crop was achieved using commercial products of *Trichoderma* and *Pseudomonas* as seed treatment and foliar sprays (Kumar et al. 2014a, b). Fungal and bacterial biocontrol agents, namely, *Trichoderma viride*, *T. harzianum*, *T. virens*, *Pseudomonas fluorescens*, and *Bacillus*

subtilis, were capable to control four seed-borne fungal pathogens of mungbean, viz., *Aspergillus niger*, *Penicillium rubrum*, *Alternaria alternata*, and *Fusarium moniliforme*. *T. harzianum* was found excellent in causing maximum growth reduction of these seed-borne fungal pathogens of mungbean, in vitro (Singh et al. 2017)

Disease management program can be improved by integrating the biocontrol agents with other management strategies. However, the inability of these biocontrol agents to survive under certain field conditions is one of the limitations. Seed treatment with bioagents in combination with half dose of vitavax (0.125%) has been found effective in controlling loose smut disease in wheat and also improved the seed yield (Gupta and Maheshwari 2007). Antagonistic potential of *Trichoderma* spp., *Bacillus* spp., and *Streptomyces* spp. was observed against *Drechslera* in different crops such as wheat, sweet corn, rice, etc. (Prasad et al. 1978; Kulkarni and Ramakrishna 1979). *Sclerotium rolfsii* when applied on seeds of wheat, barley, oats, and sweet corn increased tillering in soil infested with *Fusarium* and *Pythium* sp. *Chaetomium* spp. isolated from oat seeds protected oat seedlings from infection of seed-borne *Drechslera victoriae* (Tveit and Moore 1954). *T. harzianum* application reduced damping off in snap bean caused by *Rhizoctonia solani* (Marshall 1982). *Fusarium semitectum* and *F. sambucinum* controlled ergot in pearl millet (Thakur and King 1988). Maize seeds treated with *Bacillus subtilis* controlled seedling blight caused by *Fusarium moniliforme* and *F. roseum* (Agarwal and Sinclair 1997). The application of *B. subtilis* can also check the production of fumonisins by *F. moniliforme* during the biotrophic endophytic association and saprophytic growth with maize (Bacon et al. 2001). Seed treatment with *T. harzianum* was found to improve seedling emergence and controlled the incidence of wilt disease in gram (Gupta 2006). The effect on plant growth may be because biocontrol agents produce plant growth regulators (Kumar et al. 2017a).

25.2.4 Immunization or Resistance

This method of control focuses on planting resistant varieties. The ideal method for management of plant diseases is by using disease-resistant plants, if plants with durable resistance and satisfactory quality which are suitably adapted to the growing region are available. There is no need to undertake additional efforts to reduce disease losses by growing resistant crop varieties which is by far the most economical and reliable way for raising disease-free crops unless other diseases are also present. Resistant varieties also help in reducing the inoculum load thereby decreasing the disease intensity and thus providing spatial and temporal discontinuity against the pathogen. It eliminates environmental pollution and chemical residue effects thus protecting mankind and helping in maintaining biological balance in the environment. Resistance is achieved by altering the genetic system of the host to make it less susceptible to the disease organism.

The resistant plants are selected by growing them under high levels of disease pressure. The plants which survive this pressure become the sources of disease resistance, as they often have genetic resistance that can be utilized directly by

propagation or as sources of resistance to develop resistant plants having requisite quality characteristics of that particular crop. The standard breeding procedures of selection and/or hybridization are utilized for development of resistant plants. Hybridization procedure is used to cross a susceptible plant having the desired agronomic or horticultural qualities with another plant having resistance though may be lacking other quality characteristics as size, shape, yield, taste, aroma, etc. Mutations with x-rays or chemicals have also yielded a few disease-resistant lines. "Plant activators" are also chemicals of interest which induce plant defense responses called systemic acquired resistance (SAR) and induced resistance. Recently, resistance to the *Papaya ringspot virus* has been developed by using genetic engineering.

Breeding for disease resistance is a continuous process, but in plants having genetic resistance, selection of pathogenic races that are host differentiated is a major problem for the development of plant lines that are resistant to diseases. Whenever possible it is very important that cultivars having multiple resistance to pathogens and nematodes need to be chosen. There are two types of resistance used in plant disease management. When a single major gene provides disease resistance, it is race-specific and sometimes known as vertical resistance or qualitative resistance or specific resistance. Vertical resistance is usually not very stable as the plant line can be attacked by any new emerging pathogenic race and the resistance can be completely overcome. On the other hand, the general resistance also known as quantitative resistance is more stable and durable involving many different genes which provide resistance against pathogens. Vertical resistance provides very high-level resistance, or immunity, against specific strains of pathogens. Horizontal resistance is a lower level of resistance, or tolerance, to many more strains of disease-causing organisms. Both types of resistance are used in the development of agricultural crop plants. But disease-resistant varieties are short lived as the varietal resistance breaks down due to development of more virulent strains of the pathogen. Various strategies are being utilized to reduce the development of a race and breakdown of resistance as gene deployment methodology, where genetic monoculture is avoided by growing plant types with different genetic compositions which are interspersed on a regional basis, or ensuring that some portion of the crop plants will remain disease resistant by planting mixtures of cultivars that are genetically different.

Disease escape may take place when the susceptible plants do not develop infection due to some anatomical or physical characteristics of the plants like presence of modified stomata or thick cuticle or even leaf hairs, etc., or the environment may not be conducive for development of a disease. Usually thus, disease escape causes hindrances in the development of resistant plants against diseases. Breeding for disease resistance has been successfully achieved against the specialized diseases like rusts, smuts, powdery mildews, and viruses, but not so much against pathogens causing many blights, cankers, root rots, and leaf spots (Maloy 2005). Nowadays, there are many varieties of ornamental and vegetable crops particularly in the market which are resistant to specific pathogen. Some packets of tomato seed show a line/cv of tomato developed to be resistant to the diseases like *Verticillium* and *Fusarium* wilt (VF). Some varieties are labeled "VFN" which are also resistant to

nematodes. Recently identified varieties which are resistant to *Tomato yellow leaf curl virus* (TYLCV) and TSWV should be used in locations having high disease pressure in the past.

25.2.4.1 Tissue Culture

This technique is being utilized to study the disease mechanism, production of pathogen-free plants, and development of new disease-resistant lines in different crop plants. The meristematic tissue being free of viruses has been fully utilized for production of virus-free plantlets. Virus-free microtubers in potato have been successfully produced at CPRI, Shimla. Tissue culture is also important in the exchange of germplasm of crops like sweet potato, banana, mentha, and ginger as this approach reduces the risk of introduction of exotic pathogens.

25.2.4.2 Transgenic Plants

Biotechnology offers new opportunities for plant disease management through transfer of resistant genes into agronomically suitable genotypes. Many transgenics have been developed to control plant diseases. The coat protein-mediated resistance as in *Cucumber mosaic virus* or *Tobacco mosaic virus* in tobacco appears to be promising approach for management of viral diseases. Protection against insect pests has been achieved by inserting genes derived from the bacterium *Bacillus thuringiensis*. Such crops having inserted genes are known as genetically modified crops (GM crops). However, these crops are of concern because of associated transfer of unanticipated detrimental characteristics like unforeseen allergens to the new plants, though transmission of these unforeseen and undesirable qualities can occur through conventional plant breeding techniques also.

25.3 Management of Pathogens During Storage

The postharvest losses in India amount to 12–16 MT of food grains each year, an amount that the World Bank stipulates could feed one-third of India's poor population. The monetary value of these losses amounts to more than Rs. 50,000 crores per year (Singh 2010). Quantity losses occur when insects, rodents, mites, birds, and microorganisms consume the grain. Quality losses affect the economic value of the food grains fetching low prices to farmers (Ipsita et al. 2013). Among postharvest operations, storage is responsible for the maximum loss (7.5%). Processing, threshing, and transport cause 1%, 0.5%, and 0.5% losses, respectively (Birewar 1984).

Storage is the most important and critical postharvest operation. Crop products are eventually stored for varied periods of time depending on market demand, size of production, and the farmer's needs. Prevention of losses in food grains during storage is equally important along with the various measures undertaken to increase crop production for attaining self-sufficiency in food grains. Out of the total seed requirement in India, only about 20% good quality certified seeds are available to the farmers, and around 80% of the requirement is met up by farmers' saved seed (Raj et al. 2007; Atwal 2013; Kumar et al. 2014c). This seed is stored at local level,

and inadequate storage facilities are available with the farmers and traders. Seed moisture, storage containers, and storage environment are crucial factors that determine the seed longevity. Generally, interaction of these factors leads to contamination with microorganisms which may cause various physiological and biochemical changes in the seeds during storage ultimately leading to deterioration of seed quality especially under areas with tropical and sub-tropical conditions. Deteriorated seeds produce uneven stands, spotty fields, and fewer plants per hectare than healthy seed (Biabani et al. 2011) and with reduced growth rate (Kapoor et al. 2010).

With increasing storage duration, a significant negative correlation ($r = -0.872$) was recorded between seed germination and seed mycoflora (Gupta and Aneja 2001). Management of pathogens is essential both at pre-harvest and postharvest conditions. The storage losses can be considerably reduced by storing the seed under adequate conditions and by using safe chemicals prior to storage which effectively controls fungi and insects associated with the seed. Appropriate dressing with proper dose and proper seed coating/coverage is also important for effective seed treatment. Postharvest management of diseases through mechanical processing is another important, most economical, and new option for obtaining bunt-free grain/seed in wheat and paddy.

25.3.1 Pre-harvest Management Strategies

The deterioration of seed quality, vigor, and viability, due to high relative humidity and high temperature during the post-maturation and pre-harvest period, is referred to as field weathering (Bhatia et al. 2010). After physiological maturity, if the seeds are retained on mother plant, they will deteriorate; physiological changes in seed may lead to formation of rigid seeds or off color seeds in pulse crops (Khatun et al. 2009). Harvest delays beyond optimum maturity extend field exposure and intensify seed deterioration. Weathering not only lowers seed germination but also increases susceptibility to mechanical damage and disease infection. It is essential to follow management techniques before harvesting when the crop is still in the field to keep the crop healthy and disease-free and for the maintenance of seed health during storage. The most effective method for producing healthy seed is by sowing seed which is disease-free/certified. Healthy seed production can be undertaken in locations and in seasons where the disease incidence is almost nil. Pre-harvest foliar applications with desirable fungicides or biocontrol formulations and harvesting a properly matured crop are also beneficial in maintaining seed health during storage.

The seed may get discolored due to fungal infection in the field. Pre-harvest spraying with copper oxychloride in rice reduced grain discoloration (Govindrajan and Kannaiyan 1982). Misra and Dharam Vir (1992) observed that application of higher doses of nitrogen and phosphorus increased discoloration in paddy seed, but it decreased with large spacing in the field. Spraying of maneb at boot leaf stage, followed by a spray with common salt, was found to reduce discoloration in paddy grains effectively (Deka et al. 1996).

The fungal load on the seed is high in areas where the crop is harvested during rainy season. Indira and Rao (1968) recorded high incidence of storage fungi in seed collected from locations having high humidity. According to Misra and Kanaujia (1973), low incidence of storage fungi in some oilseeds was due to the presence of antifungal substances in the seed coat. Srivastava et al. (2012) validated this fact and checked the antifungal activity of oil extracted from *Jatropha* seed against some seed-borne fungi. Nair (1982) reported a smaller number of seed mycoflora in *Luffa acutangula* which has thick and hard seed coat and low moisture holding capacity. Susceptibility to fungal infection during storage varied with the varieties as was seen by Sheeba and Ahmed (1994) who recorded higher incidence of fungi associated with paddy seeds of high-yielding varieties as against local varieties.

25.3.2 Postharvest Management Strategies

Seed quality is highly affected by harvesting and handling methods. Mechanical damage is one of the major causes of seed deterioration during storage. Very dry seeds are prone to mechanical damage, which permits entry and easy access for microflora, making the seed vulnerable to fungal attack and reducing storage potential (Shelar 2008). Large seeded varieties are more sensitive to mechanical damage than small seeds.

Postharvest management of diseases through mechanical processing is a very important and most economical new option for obtaining bunt-free grain/seed in wheat and paddy. Karnal bunt disease of wheat caused by *Tilletia indica* is a designated disease and a limiting factor in wheat export because most countries regulate the Karnal bunt (KB) pathogen as a quarantine pest. It is difficult to detect the disease in the field, and KB-infected grain shows no symptoms until near maturity. Developing wheat kernels are randomly and partially infected due to the fungus. Infection typically occurs in only a few seeds per ear, and not all ears on a single plant are infected. The seed must be threshed and examined. Pre-cleaner and screen grader of the processing line assembly was found to eliminate 74.1% bunted seed of the total KB-infected seed present in the seed lot. Further 18 combination treatments of specific gravity separator were tested with an objective of getting maximum Karnal bunt-free seed per unit of time. Of these treatments, 2° slope of deck, 15 Kg/min of feeding, and 45 cm output deck width treatment combination further removed 21.7% bunted seed with 85.4% recovery efficiency. It was also observed that tip-infected seed is difficult to separate from the healthy seed as there is no or very little difference in density of the seed. Thus, mechanical processing could reduce KB infection by more than 90%, depending on the intensity of infection, increased the grain quality making it fit for human consumption, and also enhanced seed quality, i.e., seed germination improved by 7.76% and physical purity by 2.43% (Kumar et al. 2015). Similar results were also observed by them for paddy bunt, another seed-borne designated disease.

25.4 Conclusion

Due to global warming, the changing climate influences the dissemination of pathogens and thus the development of diseases. Indiscriminate use of inputs such as fertilizers, pesticides, water, or the cropping systems for increasing productivity has already created problems of sick soils, lowering of water table, pesticide residues, buildup of inoculum threshold levels, and resistance in the pathogens which all are harmful for sustaining our agriculture. Hence, there is an obvious need to evolve ecofriendly and environmentally safe IDM technology utilizing biological and cultural methods of disease control and to ensure proper and judicious use of chemical pesticides for sustaining healthy agriculture for the future. Thus, success of any disease management program is very much dependent on the cropping pattern, along with the aims of pest management. The key components of this program are selection of suitable areas, crop variety, water management with proper drainage, judicious nutrient management that helps in good plant growth, optimal plant density and canopy development which is conducive for proper movement of air and foliar applications, a transplanting technique with minimum transplant shock, producing seedlings in clean fields, monitoring of diseases and pests effectively during different crop seasons, and harvesting and transportation process which enhances the quality and longevity of the product. Integrated disease management (IDM) utilizes all the possible strategies like cultural practices, biological control, chemical control, use of resistant varieties, disease monitoring in the fields, etc. that are environment-friendly and give product of high quality and yield, incurring minimum cost to the farmers.

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Part IX

Mechanism of Seed Transmission and Seed Infection

Mechanism of Seed Transmission and Seed Infection in Major Agricultural Crops in India

26

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Abstract

The mechanism involved in transmission and infection of seed-borne pathogens in agricultural crops is an important aspect. Healthy tissues of seeds and vegetative propagative materials act as reservoir of major nutrients, viz. carbohydrates, protein and minerals. The seed-associated fungi and bacteria survive and proliferate by using these nutrients and, therefore, subsequently become pathogenic on seedlings and growing plants. However, seeds are also constituted with defence molecules, phenolics, lectins and many more anti-pathogenic proteins in addition to physical barriers. The successful pathogenesis depends on their aggressiveness to overcome such barriers to infect seeds. To counteract such barriers, most of the necrotrophic microbes elaborate suitable biomolecules as compared to obligate parasites of seeds. Plant viruses are exclusive biotrophic pathogens, responsible for yield and quality losses to the crop plants. The horticultural crops, viz. temperate fruits, vegetables and few plantations, are infected by such viruses. Comparatively, cereals are free from virus diseases but few localized and systemic viruses are mostly transmitted by insect vectors and are economically important. Seeds are unique as a carrier of infective virus particles. Available moisture content is normally less in well-maintained dry seeds as compared to fresh seeds from mature plants. Nutrient content is also high in seeds than foliage. These factors could favour better survival and transmission similar to seed-borne fungal mycoflora. Some of the seed-borne viruses are systemically transmitted through seeds to seedlings and adult plants. Such viruses infect male

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and female gametophytes at the time of seed formation. These processes could favour consistent association of infective viruses. Similarly, seed-borne nematodes also play an important role in seed health. However, there is less understanding about their survival, infection and mechanism of transmission. Various aspects, i.e. nature of pathogens, their mode of entry and survival, transmission and infection, are discussed in this chapter.

26.1 Introduction

Seed consists of three major components (seed coat, storage tissues and embryo) and out of these, embryos as a result of zygote play a vital role as reproductive structure. It also performs cell divisions to grow as mature plants. Storage tissues of seed are the reservoir of food mainly carbohydrates, protein and mineral nutrients. This food reservoir is important at the time of seed germination and seedling emergence. The seed coat acts as protective shield against biotic and abiotic stresses until the seed attains germination and seedling emergence. The seed coat and its major components assist seed-borne pathogens for their adherence, survival, infection and transmission to other healthy plants or seeds as a whole. However, mechanisms of infection and transmission of pathogens depend on the weather conditions and nature of crops irrespective of gymnosperm and angiosperm. The seed infection by seed-borne pathogens is entirely different from seed transmission. Seed-borne pathogens can establish successful infection and colonization in any part of seed compartment under favourable weather conditions with required inoculum potential. While seed transmission is possible by different mechanisms under favourable weather conditions. In majority of the agricultural crop diseases, true seeds or propagative materials act as a source of inoculums and, therefore, they are termed as seed-borne inoculums. The expression of disease symptoms on seedlings and adult plants by the proliferation of such inoculums and abnormal physical functions could be termed as seed-borne diseases. Different groups of microbes including fungi, bacteria and viruses are responsible for seed-borne diseases. Seed infection is mainly due to the establishment of potential pathogenic microbes in any part of the seed and this is classified based on the nature of movement (systemic or non-systemic), structural components of seed infected (seed coat, endosperms, scutellum), etc.

The seed-borne pathogens were grouped into four classes namely: (1). the pathogens for which the seed is the main source of inoculum (the disease of such inoculum is controlled by controlling seed infection), (2). pathogens in which the seed-borne phase of the disease is of minor significance as a source of inoculum, (3). pathogens never been shown to cause disease as a result of their presence on seeds and it is the largest group of seed-borne microorganisms and (4). pathogens that can infect the seed either in the field or in storage and reduce yield and seed quality (McGee 1981). These four groups of pathogens are transmitted either systemically, non-systemically or both as seed-borne or seed contaminations. However,

successful infection and transmission varies with virulence of respective pathogen and host susceptibility under suitable environmental conditions.

Plant pathogens use diverse life strategies. Pathogenic bacteria proliferate in intercellular spaces (the apoplast) after entering through gas or water pores (stomata and hydathodes, respectively) or gain access via wounds. Fungi can directly enter plant epidermal cells or extend hyphae on top of, between or through plant cells. Pathogens invaginate feeding structures (haustoria), into the host cell plasma membrane. Haustorial plasma membranes, the extracellular matrix and host plasma membranes form an intimate interface at which the outcome of the interaction is determined. These diverse pathogens deliver effector molecules (virulence factors) into the plant cell to enhance their fitness. This is a general phenomenon in almost all biotrophic and necrotrophic pathogens, which are infecting seeds and adult plants.

26.2 Defence Mechanisms in Seeds to Counteract Infection by Seed-Borne Pathogens

Seeds contain major nutrients like protein, lipids and almost all forms of carbohydrates and these nutrients are meant to support the seed germination and seedling growth. However, these also serve as nutrients for the growth and development of most of the seed-borne fungi, which are necrotrophs. The infection of seed-borne pathogens will not be successful unless the natural defence mechanisms of seeds and seedlings are defeated by the pathogen. Generally, physical structures of seeds like the seed coat, waxing and maturity standards could defend the infection by acting as barriers. Welbaum et al. (1998) found that the thick cell walls of the endosperm act as a physical barrier which in turn can slow down the penetration of pathogenic fungal hyphae. Shewry and Lucas (1997) reported that some seeds like barley contain the defence compounds, viz. thionin, endo-chitinase, ribosomal-inactivating proteins, β-glucanase, non-specific lipid transfer protein, lectin, peroxidase, thaumatin-like protein, inhibitors and α-amylase, and proteinases. Carbonero and Garcia-Olmedo (1999) reported that cereal seeds contain different types of proteinase inhibitors than legumes; perhaps due to this, seed-borne pathogens are also different in both the groups of seeds. Seeds also contain phenolics, lectins and many more anti-pathogenic proteins. Therefore, for attaining successful pathogenesis, the seed-borne pathogens should overcome all these physical and biochemical barriers.

26.3 Systemic Infection and Transmission of Seed-Borne Pathogens

A particular group of seed-borne pathogens are recognized based on their nature of infection and transmission. In this systemic group, infection takes place in any part of the plants but movement of pathogen will happen from one part to another

systemically. Successful systemic pathogens will move directly by injuries due to natural or artificial wounds or through vascular system or plasmodesmata connecting cells. Viable pathogens infect seeds by these mechanisms. For example, the halo blight pathogen of bean, i.e. *Xanthomonas campestris* pv. *phaseoli*, infects bean seeds through the vascular system, by natural openings (from the pod suture goes to the funiculus then to the raphe and tegument, or it can also happen through the micropyle).

26.3.1 Systemic Infection

The active association of fungal pathogens with seeds is the common phenomenon in systemic infection. Generally, seed infection is the establishment of a pathogen within any part of a seed, which may occur systemically, either through vascular system or plasmodesmatic connections or directly through floral infection or penetration of the ovary wall, seed coat or natural openings. Fungal pathogen infects through flower, fruits or seed stalks or penetration through stigma (*Sclerospora graminicola*, *Ustilago nuda*, *Ustilago tritici* and *Claviceps fusiformis*); infection through ovary wall or seed coat (*Colletotrichum lagenarium* in watermelon and *Cercospora kikuchii* and *Colletotrichum truncatum* in soybean); natural openings or injuries (*Cercospora sojina*) enter through pores and enter through hilum (*Alternaria sesamicola*). However, infection of any one seed may take place by more than one process (Figs. 26.1a and 26.1b). For some seed-borne pathogens, stigma acts as cushion to lodged spores and subsequently same spores germinate with available moisture over stigma. Active hyphae from germinated spores reach the ovary through style and infect the same via ovary wall and further proliferate. In some cases, pathogenic mycelia remain as dormant asexual structure and become active whenever respective seed germinates.

Fig. 26.1a Systemic infection and upward transmission of *C. truncatum* into cotyledons of soybean seed

Fig. 26.1b Systemic infection and upward transmission of *U. nuda* and *U. tritici* into spikelets of barley and wheat seeds and *F. oxysporum* f.sp. *vasinfectum* from cotton seeds

26.3.1.1 Systemic Infection Through Stigma, Ovary Wall, Natural Openings and Wounds

Systemic infections to seeds occur through flower, fruit and seed stalks. Seed-borne pathogen could first enter into flower/fruit stalk and pedicel/peduncle and seed stalks and funiculus. For example, the vascular wilt of water melon incited by *Fusarium oxysporum* f.sp. *lagenarium* invades seeds from vascular bundles of fruits. Similarly, cotton wilt pathogen i.e. *F. oxysporum* f.sp. *vasinfectum,* and *Septoria glycines* of soybean systemically enter into seeds through mother plants. Linseed anthracnose caused by *Colletotrichum lini* also penetrates seeds through capsule from infected plants. Mycelia may penetrate the fruit stalks, capsule and, finally, seed coat and in the same crop, *Septoria linicola* infects from the fruit stalks, then capsule, placenta from funiculus and seed coat. The pathogens like *Fusarium oxysporum* f.sp. *pisi*, *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium oxysporum* f.sp. *moniliforme* and *Septoria glycines* are some of the seed-borne pathogens, which are systemically transmitted through infected mother plants into seeds. Downy mildew of sunflower (*Plasmopara halstedii*) and sugar beet wilt (*Verticillium dahliae*) are also transmitted though floral parts of the plants into seeds.

Systemic infections are observed in loose smut diseases of barley and wheat (*Ustilago nuda* and *U. tritici*) and leaf blight of sweet pepper caused by *Alternaria alternata*. In the case of seed-borne viruses, they infect through pollen, the male gamete carries the virus and when joining the ovule, it generates an infected embryo. If both the male and female gamete are infected, they can even produce an infected endosperm (Nome et al. 2002). The resting spores of fungal pathogens lodge on the stigma, germinate under favourable conditions, penetrate the ovary and reach the

embryo. In the case of loose smut pathogens of wheat and barley, the teliospores fall on stigma and germinate. The promycelium from germinated spores passes through the wall and other tissues until it finally reaches the embryo. In some cases, the natural openings of the plants and seeds in particular favour the systemic infection. Natural openings like the hilum and the micropyle or wounds generated during threshing are spots or entry points, wherein pathogens like *Xanthomonas campestris* pv. *phaseolicola* in bean and *Pseudomonas syringae* pv. *lachrymans* in cucumber can enter for systemic movement and infections (Nome et al. 2002).

In fleshy fruits like cucumber, melon, eggplant, tomato, sweet pepper and others, contamination occurs directly through the funiculus or in the tegument, during the process of seed formation. Seed-borne pathogens, i.e. *Colletotrichum lagenarium* in watermelon and *Rhizoctonia solani*, are best examples, when they infect and colonize in fleshy fruits. These pathogens are capable of infecting from the placenta and penetrate to the developing ovule or seeds which will be under formation without lignifications on cover (Agarwal and Sinclair 1996).

Seed-borne pathogens penetrate and infect the ovary via stigma. Fungal pathogen's hyphae follow the path of pollen. In this case, first spores are lodged on stigma and germinate. Germ tube from spores enters the style, infects the ovary and establishes in developing seeds (loose smut of wheat – *Ustilago tritici*, *Ustilago nuda*, *Sclerospora graminicola* and *Claviceps fusiformis*). In some cases, the pathogen gains entry through the ovary wall and seed coat. Batts (1955) reported that the loose smut pathogen causes infection through stigma at flowering time. The infective diploid hyphae penetrate between stigma papillae and grow intercellularly through the style into the ovary. The germinating teliospores penetrate directly through the ovary wall and grow until they reach the embryo and the embryo secretes chemostatic substances that attract the hyphae towards the embryo. The embryonic infection takes place through the ovary wall. Teliospores germinate and form dikaryotic promycelia (hyphae) which penetrate the ovary wall and pass along integuments of grain and cross the endosperm. Profuse infective hyphae are formed in the hypocotyl region after entering into the scutellum and embryo; and, finally, intracellular hyphae are formed on the pericarp, testa, aleurone layer/endosperm, scutellum and embryo of seeds (see flow chart). Seed germination and seedling health are poor from smutted seeds of wheat and embryo bound inoculum could express complete symptoms during adult plant stage (Chart 26.1 and Figs. 26.2a, 26.2b, and 26.2c).

Out of different cereal crops, rice, wheat, maize and millets are widely grown in India in different agroclimatic regions; the mechanisms of infection and transmission of seed-borne pathogens differ in response to host species and nature of pathogens. Almost in all the crops, seed-borne fungal pathogens are predominant followed by seed-borne bacterial diseases and virus diseases are significantly less in all these crops. The quantitative estimation of economic losses due to seed-borne pathogens in these crops is inconsistent and varies between seasons, crops and variety, variability in the rate of transmission and infection of seed-borne pathogens in these crops. Therefore, the comprehensive account on these aspects is furnished on major seed-borne pathogens which are important to Indian agriculture.

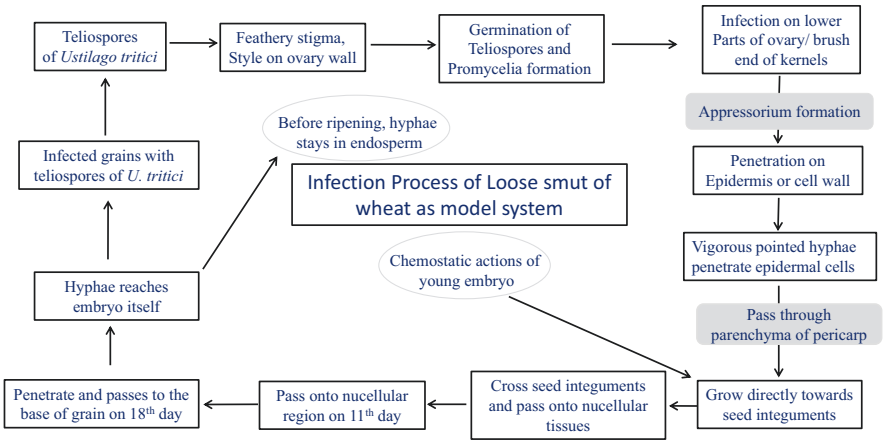

Chart 26.1 Infection process of loose smut pathogen in wheat

Fig. 26.2a Wheat seeds inoculated with smut teliospores are poor in germination and growth under field conditions

Fig. 26.2b Systemic and localized transmission and severe infection in spikelets from infected seeds of wheat

Fig. 26.2c Honey bees also play a role in transmission of smut spores from grasses and other main hosts

26.4 Non-systemic Infection

This type of infection occurs through the ovary wall, pericarp and integuments of seed coat. The loose smut of wheat is the best example for this type of infection. In another type, fungal pathogen penetrates during different developmental stages of a fruit/pod. They invade maturing seeds (anthracnose of bean incited by *Colletotrichum lindemuthianum* and *Ascochyta pisi* of pea). In the case of linseed anthracnose, the pathogen (*Colletotrichum linicola*) penetrates through drying petals and maturing capsules. Pathogen *Rhizoctonia solani* penetrates through the pericarp of capsicum and enters further through funiculus into the embryo. Similarly, *Sclerotinia sclerotiorum* of crucifers penetrates in drying petals which provides foodhold for mycelia invasion of wind-borne ascospores. Most of the leaf blight pathogens which are transmitted through seeds spread and infect in non-systemic means. Atmospheric weather factors like low temperature, dew deposition and wind current are crucial factors for such transmission and infection on foliage and subsequent colonization in seeds. The leaf blight of wheat caused by *Bipolaris sorokiniana* is the best example that the seed-borne conidia germinate and colonize on foliages of seedlings and adult plants and the environmental conditions favour further spread and infection in the field (Fig. 26.3 and Chart 26.2).

Fig. 26.3 Rapid infection of *B. sorokiniana* on foliage

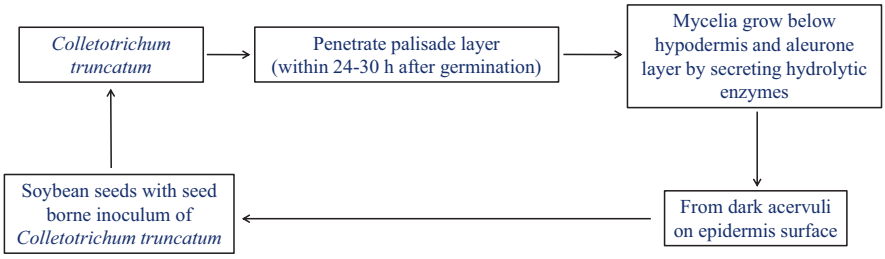

Chart 26.2 Infection process of non-systemic seed-borne pathogen

26.5 Seed Contamination or Infestation

Seed-borne pathogens are also transmitted by infestation or contamination. This is passive association of pathogen with the seeds. Pathogens adhere to surface or are mixed with seeds at anytime during harvest, extraction, threshing or processing until packing of seeds in suitable containers. The seed concomitant contamination will take place with pathogenic structures, mixed with infected plant parts and soil as well. Fungal pathogens carried on the seed coat surface transmitted by means of seed contamination or infestation are like *Alternaria brassicae* and *A. brassicicola* (cruciferous), *Alternaria linicola* (flax), *A. longipes* (tobacco), *A. radicina* (carrot), *Ascochyta pinodella* (pea), *Ascochyta rabiei* (gram), *Drechslera sorokiniana* and *D. oryzae* (rice), *Pyricularia oryzae* (rice), *Protomyces macrosporus* (coriander), *Rhizoctonia solani* (eggplant), *Colletotrichum graminicola* (pearl millet), *Sclerospora graminicola* (finger millet), *Peronospora manshurica* (soybean), *Phytophthora phaseoli* (bean), *Neovossia indica*, *Urocystis agropyri* and *Tilletia caries* (wheat), *Ustilago hordei* (barley and oat), *U. maydis* (maize), *Sphacelotheca reiliana*, *S. cruenta* and *S. sorghi* (sorghum), *Puccinia carthami* (sunflower), *Melampsora lini* (linseed) and *Uromyces betae* (beetroot). Some pathogens gain entry directly into the ovary and become seed-borne. Malik and Batts (1960) reported that infective hyphae of covered smut of barley (*Ustilago nuda*) enter directly into the ovary but there will not be any indication about the presence of hyphae on the style. The flag smut of wheat, which was considered as minor disease has also become the major problem in some pockets of wheat-growing regions in India. The pathogen (*Urocystis tritici* Koern/*U. agropyri*) infects mostly leaf tissues. Severely infected plants form shrivelled grains. Spores of this pathogen are both seed- and soil-borne and after germination, they infect seedlings and reside in apical cells to become systemic. Infective hyphae grow both inter- and intracellularly and symptom expressions occur in any growth stage based on weather conditions. Another important seed-borne disease, i.e. hill bunt or stinking bunt, incited by *Tilletia caries* (DC) and *T. foetida* is very common in northern hills of India. This pathogen is a typical example of seed contamination in which teliospores are carried on seeds as a source of inoculum in addition to inoculums present in soil as a primary source. The smutted seeds contaminate healthy seeds and such seeds favour germination of spores in soil. After germination, uninucleate primary sporidia are formed on a basidium and binucleate secondary sporidia are formed as 'H'-shaped structures. The secondary sporidia which are capable to infect seedlings are dikaryotic in nature; otherwise seedling infection will fail. Profuse colonization of inter- and intracellular tissues takes place and pathogen becomes systemic during grain formation. Infective mycelia grow with spikes and reside in kernels; subsequently, matured mycelial cells get transformed into resting spores known as teliospores. These spores are carried with grains or shattered on soil surface to act as the primary inoculum for subsequent crop.

Mitra (1931) first reported Karnal bunt caused by *Neovossia indica* (Mitra) from Karnal (Haryana). The symptoms appear only after the grains are formed. Smutted seeds are pale in colour and irregularly distributed on a spike. Black powdery mass

of teliospores are disseminated when they rupture and fall on soil or adhere to seeds as contaminant. Germinated spores produce sporidia and these are transmitted to floral parts by wind and subsequently enter into the ovary wall. Though the infective mycelia can enter into cells of the ovary and produce teliospores (dormant spores), systemic infection is not reported like in the case of other smuts.

Overall, the infection and establishment of seed-borne pathogens depends on the seed structures. The indehiscent fruits (Gramineae) attract mainly the fungal pathogens but the dehiscent fruits (mostly legumes) attract mainly seed-borne bacteria and viruses. In this type, the vascular connection is strong with mother plants and seeds. Since, the large cotyledons have reservoir of nutrients and other minerals, they favour seed-borne bacteria and viruses and the pathogen can move through the vascular system. In some cases, the type and orientation of flower arrangement on a plant also influence the infection and transmission of seed-borne pathogens. For example, the closed flowers have less chances of infection by loose smut pathogen (*U. tritici*) as compared to open flowers present in wheat. At cellular level, the greater the size and number of cytoplasmic connections, the higher the embryonic seed infection in vascular pathogens. In the case of legumes, more cytoplasmic connections are present as compared to cereals, therefore legumes have higher percentage of bacteria and virus infection.

Some of the successful infections of seed-borne pathogens also depend on the location of inoculums in seeds. Seed-borne pathogens, which are specifically located in the embryo, are *Alternaria alternata* (sunflower), *A. triticina* (wheat), *A. padwickii* (rice), *A. sesamicola* (sesame), *A. brassicicola* (cabbage), *Colletotrichum graminicola* (sorghum), *Fusarium oxysporum* f.sp. *cumini* (cumin), *Pyricularia oryzae* (rice), *Sclerospora graminicola* (sorghum) and *Ustilago tritici* (wheat). The endosperm and perisperm also play a critical role in infection and transmission of these pathogens. Similarly, the seed coat and glume are also important for colonization, infection and transmission of seed-borne fungal pathogens.

26.6 Mechanisms of Transmission and Infection of Major Seed-Borne Pathogens of Rice

The IRRI classified the seed-borne fungal pathogens of rice into four categories, namely, a. pathogens which invade both hulls and kernels (the best examples are the seed-borne inoculums of *Pyricularia oryzae* and *Fusarium moniliforme*, which are responsible for seedling diseases of rice), b. fungal pathogens that invade the same parts (hulls and kernels) but result in kernel discolouration with low quality of seeds or grains (pathogens involved in this class are *Alternaria padwickii*, *Curvularia lunata/C. intermedia* and *Bipolaris sorokiniana*), c. seed-borne fungal pathogens (*Ustilaginoidea virens* and *Tilletia barclayana*) that infect and produce spore mass on infected spikelets which results in problems during processing and d. pathogens that infect only hulls, responsible for reducing commercial value by producing discolouration of grains (*Nigrospora oryzae*, *Fusarium* spp., *Aspergillus* spp., *Diplodia oryzae* and *Septoria* spp.) (Figs. 26.4a and 26.4b) (Kato et al. 1987).

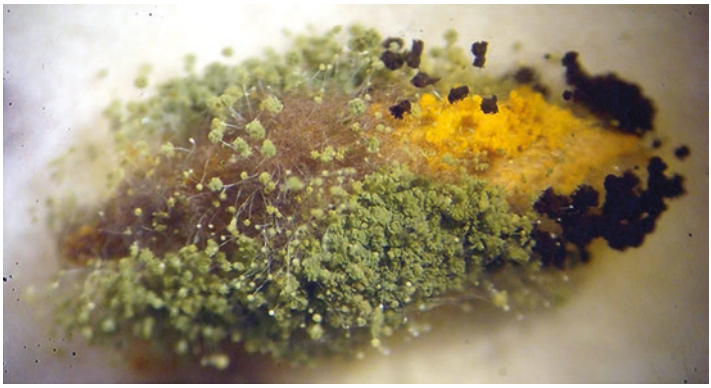

Fig. 26.4a Saprophytic fungi masked seed-borne inoculum of *B. oryzae*

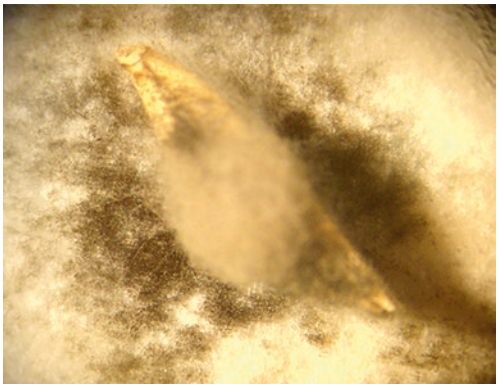

Fig. 26.4b Rapid proliferation of *B. oryzae* from seeds

26.6.1 Rice Blast

This disease incited by *Magnaporthe oryzae* is reported from more than 85 countries (Kato 2001). Skamnioti and Gurr (2009) reported that about 10 to 30% of the annual rice harvest is lost due to infection by the rice blast fungus. The primary infection of *P. oryzae* produces rice blast symptoms which occur in empty glumes, pedicels and the distal ends of hulls (Hirano and Goto 1963). The air-borne conidia infect rice spikelets soon after heading, and conidia produced on spikelets serve as secondary inoculum for further infection of the panicle (Kato et al. 1970). Manandhar et al. (1998) was first to take up quantitative study on transmission and infection of rice blast pathogen. A significant linear relationship on a logistic scale was recorded between neck blast symptoms and seed infection. This relationship was further demonstrated when seeds were collected from diseased secondary branches of panicles. A 10% increase in neck blast could result in almost double the level (22%) of increase in seed infection. Seeds collected from secondary grain-bearing branches

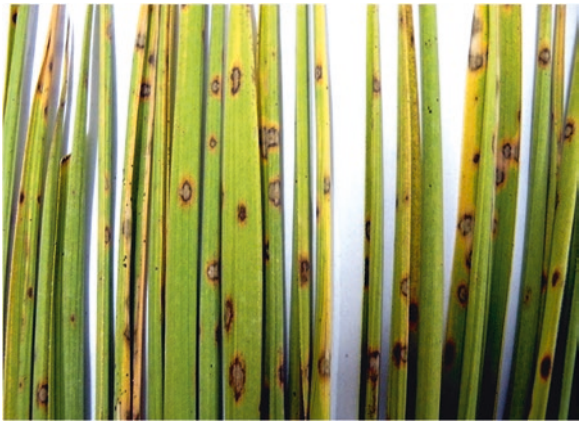

Fig. 26.5 Infection of *M. oryzae* on paddy leaves

of panicles with symptoms had a significantly higher degree of infection by the pathogen than apparently healthy branches. Seeds from panicle having neck blast symptoms are subjected to higher degree of infection than from seeds of apparently healthy panicles. In general, matured or filled grain will have lower degree of infection than unfilled grains perhaps due to less chance to take aggressive infection by the pathogen. It is well documented that the embryonal end of the living seed of paddy is highly susceptible to infection by the pathogen, since the embryonal tissues have high protein content, which could possibly enhance the sporulation of the pathogen. When the seeds are dead, the fungus can utilize the whole seed as a substrate (Limonard 1968). Rice leaves with severe infection favour profuse sporulation and disseminate the same for subsequent infection on healthy leaves and other parts of rice crop under field conditions (Fig. 26.5).

26.6.1.1 Physical and Molecular Mechanisms Associated with *M. oryzae* Infection in Rice

In brief, the rice blast pathogen is hemi-biotrophic, having the distinct infection strategies. Pathogen follows its initial infection process similar to the developmental process of most of the fungal pathogens of crop plants. Viable conidium after germination starts its initial proliferation inside living host cells before switching to a destructive necrotrophic mode on dead cells. Conidia after germination produce a germ tube and from that a differentiated and specialized infectious structure known as appressoria is formed to adhere on the surface of host tissues using mucilage. Howard et al. (1991) and Ribot et al. (2008) reported that because of enormous turgor pressure generated by the pathogen inside its melanized appressorium, a thin penetration peg pierces the host surface using this pressure and enters into leaf epidermal cells. After penetration, infection pegs differentiate into infectious hyphae both intracellularly and intercellularly (Heath et al. 1990, 1992). In response to biochemical and molecular interactions, blast lesions appear at infection sites (Tucker and Talbot 2001). Dean et al. (2005) sequenced the entire genome of *M. oryzae* and Jeon et al. (2007) identified hundreds of genes involved in its pathogenesis by using functional genomic approaches.

Physiological and molecular analysis of paddy seed and seedlings in response to *M. oryzae* infection unfolded the relationship and factors responsible for defending infection or in other words as resistance. The seed health could be maintained and protected by augmenting such factors associated with seeds and adult plants. Cho et al. (1998) isolated and identified two antimicrobial substances (4-hydroxybenzoic acid and trans-4-hydroxycinnamic acid by LC-MS and ^{1}H- and ^{13}C-NMR) from rice hull. These two substances had different inhibition profiles against various microorganisms. Most of the bacteria were sensitive to trans-4-hydroxycinnamic acid and 4-hydroxybenzoic acid. Bagnaresi et al. (2012) reported that the gene ontology (GO) enrichment in compatible and incompatible interactions of *P. oryzae* and its host was similar but recorded dissimilarity in the gene sets contributing to each gene ontology. The genes related with phytoalexin biosynthesis, flavin-containing monooxygenase, chitinase and glycosyl hydrolase 17, were dramatically up-regulated. The resistance reactions expressed drastic changes and up-regulation of several fungal genes encoding secreted effectors, which may be involved in the initial infection process. This information provides clues for understanding the host immune response against *M. oryzae* attack and the host defence (Kawahara et al. 2012). Subsequently, Wei et al. (2013) studied the transcriptome changes in blast susceptible and resistant rice cultivars in response to rice blast fungus infection at 24-h postinfection and documented that the transcriptional profiles of rice between both the categories of rice cultivars are mostly similar.

Wang et al. (2014) performed a comparative transcriptome analysis in early and later interaction stages of *M. oryzae* with rice and a total of 608 genes were found to have multiple biological functions, such as cell signalling, redox states and proteolysis. Out of these, 18 genes were identified and validated for encoding receptor-like kinases. Out of 608 genes, 231 genes were more highly accumulated in resistant type of interaction, indicating that these genes play a major role in the response against *M. oryzae* infection. This kind of information helps to understand interactions in both early and later stages of the infection which in turn will be useful in seed health testing and formulating molecular standards which may be useful in seed trade and certification programme.

26.6.1.2 Transmission of *M. oryzae*

Rice blast pathogen gets transmitted from infected seeds to seedlings and the blast lesions appear first on the second or third leaf of seedlings emerged from respective infected seeds in about 3 weeks after seeding. Seedling infection with symptoms as lesions could be seen at a temperature ranging between 25 and 28 °C (Manandhar et al. 1998). The seed transmission of pathogen from infected seeds to seedlings depended on the degree of covering by pathogen on the seeds (no covering, light covering and complete covering). Obviously, light and complete covering of infection on seeds resulted in low level of seedling infection than no lesion coverage on seeds. They further confirmed significant differences between light covering and complete covering of seed infection and that former results in significantly higher seedling infection than later category, i.e. complete covering (Manandhar et al. 1998).

According to IRRI, the primary infection of *P. oryzae* occurs in empty glumes and pedicel. Infective hyphae penetrate outer surface of hulls, reach the hypodermal tissues within 24 h of infection and reach parenchymatous tissues within the next 24 h. After rapid proliferation, hyphae spread all over the tissues vigorously within the next 24 h with profuse growth in the lemma and palea. The conidiophores and conidia grow on large hairs and trichomes which are in contact with hypodermal tissues at the base of spikelets. The proliferated inoculums spread into the radicle and coleoptile region but survival and infection in the former case will be doubtful because of high moisture content of soil coupled with other physical conditions which could be unfavourable. However, inoculums spread into the coleoptile region serve as a primary source of inoculum for seedling infection and further dissemination of the pathogen (Kato et al. 1987).

26.6.2 Brown Spot

Brown spot of rice (*Oryza sativa*) caused by *Bipolaris oryzae* (Breda de Haan) Shoem. Syn. *Drechslera oryzae* (Breda de Haan) Subram. & Jain, *Helminthosporium oryzae*, teleomorph: *Cochliobolus miyabeanus* (Ito & Kurib) is an economically important seed-borne disease in India. This disease was first described in 1900 as *H. oryzae* and the teleomorph was found in Japan. Pathogen causes necrotic spots on seeds and grain discolouration resulting seedling blight. In general, infected seeds serve as a major source of inoculum, resulting in serious loss in rice production in India. This disease was reported to be the major cause for the famous Bengal famine in India during 1942. Rice yield loss was ranging from 40% to 90%. Prabhu et al. (1980) estimated grain weight loss from 12 to 30% and 18–22% loss in filled grains. Brown spot of rice is caused by the fungus *D. oryzae* (B. de Haan) Subram. & Jain; earlier it was known as *H. oryzae* Breda de Haan. However, the seed-borne nature of the pathogen has been designated as *B. oryzae* since 1959. This species is economically important for seed quality and trade, because the potential inoculum resides in any part of the seed and causes severe infection during germination and further transmission. Infected plant parts especially leaf tissues, stem and neck portion result in indirect loss to the crop and diseased seeds, which are unfit, do not fetch good market price. Direct loss is mainly because of physical appearance and poor quality of rice after milling. Therefore, the seed infection and transmission are more significant than infection in seedlings and adult plants other than seeds. Dormant mycelium is present in the seed coat, pericarp, endosperm and sometimes in glumes. Brown stain is observed on infected grains with mass of conidia as dark brown or black under severe infection. Primary infection is possible from infected seeds but not all seeds may transmit the pathogen to seedlings. Thomas (1940) reported that the infection might also take place from soil. Inoculum from the seed integuments get transmitted to coleoptiles but subsequent transmission to seedlings and adult plants is still doubtful even under favourable conditions. Suzuki (1930) reported that brown spot pathogen can survive in the form of dormant mycelia inside the seeds even up to 4 years; however Padmanabhan (1953) found that the

pathogen can survive as primary inoculum up to 1 year or till the next growing season. Seed germination is significantly reduced in infected seeds than healthy seeds and pathogen was detected in 60% of abnormal seedlings and 78% of seedlings with root and shoot decay (Guerrero et al. 1972). The secondary infection and transmission of pathogen is possible by air-borne conidia from lesions produced on leaves and different parts of infected plants (Figs. 26.6 and 26.7).

Schwanck et al. (2015) reported a significant effect of seed-borne inoculum for the area under the disease progress curve of brown spot incidence and severity. Seed-borne inoculum levels of *B. oryzae* did not affect final severity and kernel infection, which was not correlated with each other. Plant population density was significantly reduced with the increase of seed-borne inoculum levels. They concluded that the seed-borne inoculum levels did not affect yield although significantly reducing plant population, which may be due to the rice having a low population, can be compensated through tillering. The risk of yield loss by sowing *B. oryzae* infected seeds was low but the early onset of the disease by increased

Fig. 26.6 Transmission of *B. oryzae* from seeds and seedlings to adult plant

Fig. 26.7 Brown discolouration on infected seeds due to *B. oryzae*

levels of seed-borne inoculum was dependent on seasonal weather conditions. Infected seeds carry the pathogen's inoculum for fresh infections and transmission from nursery to adult plants. Infected seeds are discoloured and shrivelled. Rice after milling from such grains is unfit for consumption. Therefore, knowledge on the mode of infection and transmission will help to formulate suitable management strategies. The fungal pathogen is soil- and seed-borne and the infected seeds harbour dormant mycelia and conidia as carryover inoculums from one season to another, in addition to leftover stubbles and debris of infected plants in paddy field. Conidia germinate at optimum temperature but high temperature above 30 °C may be inhibitory for spore's germination and successful infection. The germ tube from conidium attaches to young apical cells of emerging primary leaf from seeds but secondary leaves from top are highly infected than top most leaf which is normally resistant perhaps due to biochemical barriers. Different workers reported that the thick epidermal cells and cuticular layers are resistant to penetration of *B. oryzae*. It is reported that silicate epidermal cells are less prone to pathogen infection (Wang et al. 2017). After appressorium formation, infective hyphae penetrate epidermal tissues and proliferate for colonization and symptom expression by various physical, biochemical and molecular events. It is reported that penetration of infective hyphae can happen in healthy tissues through stomata openings. Availability of free water for about 6 h is essential for disease development. The cloudy weather with temperature range of 16–24 °C and humidity favour rapid infection and colonization of the pathogen on tender seedlings. Nursery beds under continuous moisture and humid conditions are more prone to epidemic of this disease than dry conditions. When the inoculums in the form of dormant mycelia and infective conidia are deep-seated, seed treatments are not effective. This was evidenced from our laboratory experiments that the apparently healthy seeds were not able to germinate for long period during the seed health testing under blotter method. However, proliferation of mycelia of *B. oryzae* was very slow initially and very rapid when those seeds were incubated under optimum conditions required for pathogen's multiplication (Figs. 26.8, 26.9, 26.10a, and 26.10b). Seeds of susceptible varieties carry the inoculums as seed-borne in any part of the seeds to nursery beds and when such seeds

Fig. 26.8 Conidia of *B. oryzae* from seeds

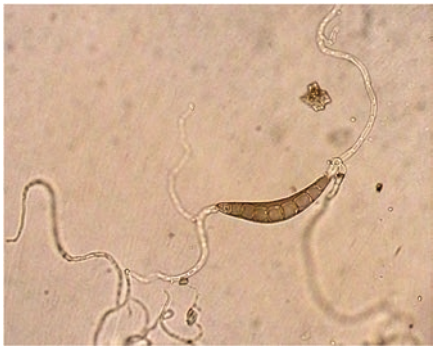

Fig. 26.9 Infective hyphae from both ends of *B. oryzae* conidium

Fig. 26.10a Seed-borne inoculum of *B. oryzae*

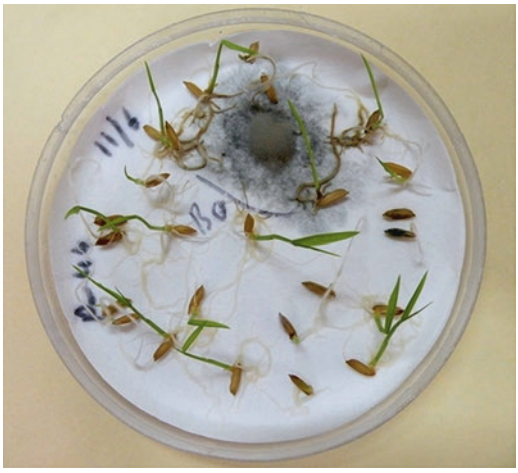

Fig. 26.10b Seed-borne inoculum of *B. oryzae*

are sown deeply, seedling emergence will slow down and pathogen has the opportunity to sufficiently multiply and infect seedlings resulting in pre-emergence loss.

Bipolaris oryzae is a common rice seed pathogen, causing yield reduction and seedling blight or weakening of the seedlings (Malavolta et al. 2002). The knowledge of the atmospheric concentration of *Pyricularia* and *Bipolaris* air-borne spores together with a correct examination of the crops can provide information about the risk of infection during the vegetative season, thus allowing for a more accurate use of fungicides on rice crops, according to the modern concept of integrated control. Picco and Rodolfi (2002) reported the air-borne mechanism of *P. grisea* and *B. oryzae* spores, the causal organisms of blast and brown spot in rice field. *B. oryzae* was detected at the end of June, reaching its peak in July. Brown spot symptoms in-field were detected 6–7 days after the aforementioned peak. *P. grisea* was monitored later than *Bipolaris*. Rice seeds with different incidence levels of *B. oryzae* were obtained by artificial inoculations and evaluated on physiological quality, seed-seedling transmission and production parameters. Seed germination reached the highest level at the lowest incidence. The number of dead or infected seedlings, at 17 days post-sowing, was highest at the highest incidence levels of the pathogen, revealing damping off as a result of infection. The pathogen recovery from seedlings, variable from 1.0% to 7.2%, was directly related to its incidence level on the seeds. No significant differences in the seed-seedling transmission (average 18.6%) were observed among the distinct seed incidence levels.

Transmission of brown spot pathogen under field conditions indicated that the rate of seedling emergence was inversely related to seed incidence, but no significant differences in the yield were observed among the treatments. Significant correlation coefficients were observed between seed incidence of *B. oryzae* versus seed germination, sterilized soil emergence, dead or infected seedlings, pathogen recovery, field emergence and one hundred panicle weight. Therefore, seed transmission of brown spot from seeds is important than the air-borne conidia.

26.6.3 Stackburn of Rice

Stackburn disease in rice is caused by *Alternaria padwickii* and loss will be more in seeds than other plant parts of rice. Padmanabhan (1949) recorded 51–76% and Cheeran and Raj (1966) recorded up to 80% loss in India. Symptoms appear on leaves, seedlings and grains. Infected seeds transmit the pathogen into coleoptiles and radicals showing necrotic spots and seedlings died under severe infection. Infection reaches to kernel causing kernel spots, discolouration and shrivelling of seeds. Ou (1985) reported that the pathogenic propagules in the form of dormant mycelia and spores are located in tissues on the endosperm, embryo, glumes and bran layers. Infected seeds fail to germinate because of rotting of seeds, coleoptile and root. Inoculum located inside the seeds acts as a primary source of infection.

Fig. 26.11 Systemic transmission and field symptoms of Bakanae inoculum of *F. moniliforme* from soil or seed

26.6.4 Bakanae (*Fusarium* Wilt)

The bakanae or *Fusarium* wilt of rice is widespread in India. Ou (1985) recorded 15% yield loss due to this disease. This disease is incited by *Fusarium moniliforme* (Sheld.) (teleomorph, *Gibberella fugikuroi*) which induces profuse tillering in single plant with internode elongation perhaps due to production of gibberellic acid and fusaric acid (Fig. 26.11). The abnormal growth of infected seedlings or adult plants than the normal plant's height is known as 'bakanae', in which severely infected seedlings die either before or after transplantation, based on their infection intensity and inoculum potential of test pathogen. Systemic infection of the pathogen could transmit from collar region to stem, inflorescence and all other floral parts of plants such as the glume, pedicel, palea and lemma (Hino and Furuta 1968). However, pathogenic propagules in the form of dormant mycelia or conidia in the embryo are very critical for systemic transmission to seedlings and adult plants. In most of the susceptible varieties, the deep-seated inoculums present in embryo kill the embryonic tissues of seeds which result in failure of seed germination even under normal conditions. The apparently healthy seeds could result in seedling infection even up to 31% (Kanjanasoon 1965).

26.6.5 Kernel Bunt

This is a designated disease in India. Symptoms of this disease can be noticed only on grains at the time of maturity. Most of the seeds are infected either completely or partially but the presence of bunt spores is the common symptom of this disease. Appearance of minute black pustules or streaks with black spores by busting the grain is the characteristic symptom expressed by the pathogen. Shetty and Shetty (1986) reported that the seeds with teliospores or loose spores on the seed coat facilitate for early transmission. The spores fallen on soil surface germinate and

produce the primary and secondary sporidia (Figs. 26.12a, 26.12b, and 26.12c). The secondary sporidia forcibly discharge and disseminate for fresh infection, which was confirmed from the floral parts by Chowdhury (1946). Ou (1985) documented that the teliospores can be viable up to 3 years in stored grains and 1 year in normal conditions. Duhan and Jakher (2000) recorded an incidence of kernel bunt across different rice cultivars, districts and years ranging from 0.05% to 1.20%, in various districts of Haryana. A total of 8.33% of the seed samples were rejected due to bunt

Fig. 26.12a Field symptoms of kernel bunt in rice grains

Fig. 26.12b Shedding of bunt spores for transmission

Fig. 26.12c Bunted seed of paddy

infection in 1993, failing to attain minimum seed certification standards; rejection was negligible in 1994 and 1997 with 1.19 and 0.07% of the samples being rejected and there was no rejection of seed samples in 1995 and 1996.

26.6.6 False Smut

The rice false smut caused by *Ustilaginoidea virens* is an important constraint affecting rice yield and quality in Asia (Zhou et al. 2008). Mandhare et al. (2008) recorded an incidence up to 78% from the seed samples of commercially cultivated varieties of paddy from Maharashtra state. Nallathambi et al. (2010, 2012a, b) also reported false smut endemic in northern India. Among rice diseases, false smut incited by *Ustilaginoidea virens* (Cooke) Takahashi emerged as a major constraint in productivity and quality seed production of commercial varieties and hybrids grown in India (Nallathambi et al. 2013). Nallathambi et al. (2013) concluded that the concomitant contamination of chlamydospores of *U. virens* could drastically reduce germination and other seed quality parameters of commercial varieties in India. In addition, the rate of milled rice decreases as the number of false smut-infected grains increases (Ding et al. 1997).

False smut of rice can produce toxins which can inhibit seed germination and embryo bud growth (Wang et al. 1998). Out of a total of 609 rice seed samples collected from various districts of Haryana, the incidence of false smut across different rice cultivars ranged from 0.05% to 0.50% (Duhan and Jakher 2000). Hegde and Anahosur (2000a, b) reported that chlamydospores of false smut pathogen collected from an infected rice inflorescence remained viable for up to 4 months when stored at room temperature (25–35 °C), while those stored in the refrigerator (4–6 °C) retained their viability for up to 7 months. Pseudosclerotia retained their viability up to 7 months at room temperature (25–35 °C) and in paddy straw at 25–40 °C, and for 9 months in the refrigerator (4–6 °C). They recorded the presence of true sclerotia; and their germination through stromatic head bearing ascospores, which was observed for the first time in Karnataka, India. Seedlings emerged from seeds of

Fig. 26.13 Air-transmitted ascospores infection and smut sclerotia formation on seeds

infected panicles had poor seedling vigour, and reduced root length was more pronounced than reduced shoot length (Fig. 26.13). The disease had significantly increased chaffiness (up to 40.9%) and decreased 1000 grain weight (up to 32.4%) and panicle weight (up to 25.5%). Bhagat and Prasad (1996) reported that *U. virens* was favoured by increased humidity resulting from frequent irrigation.

Nallathambi et al. (2010) collected representative samples (65 Nos) of rice false smut (*Ustilaginoidea virens*) and analysed them for their disease severity and diversity in India. Prominent variation in morphogenesis was recorded among the pathogenic isolates from different agroclimatic regions. The structural size and location of smut balls in panicles were variable in response to rice genotypes and their cultivation area. Tsuji (2001) also studied the seed transmission of *U. virens* in rice. Mulder and Holliday (1971) described that false smut of rice appears as olive green, velvety, globose masses, up to 1 cm diam. in some of the ears of the inflorescence. The spore ball, beneath the dark layer of mature spores, is orange yellow, paling inwards until it is almost white and as it ages it becomes almost black. Nallathambi et al. (2010) observed diversity in ornamentation of smut balls in infected panicles and the intensity was variable under different rice ecosystem in India. Mulder and Holliday (1971) suggested no evidence for seed transmission and the conidia are probably viable for a short time only. The air-borne conidia have a diurnal periodicity with peak at 22 h, numbers being very low between 4 and 16 h (46 and 316). The ascospores of the reported perfect stage may also be air-dispersed and responsible for successful infection. The number of cloudy hours during the day was positively correlated with false smut development (Bhagat et al. 1993). Fan et al. (1996) studied the biological characters and some uncertain key points in relation to infection cycle of rice false smut. The optimum temperature for chlamydospore germination was 28 °C, pH range from 5.8 to 6.3. In addition, sugar and light enhance chlamydospore germination. The germination ability of chlamydospores and sclerotia stored in the field was higher than those stored inside. Germination rates coincided with the infection date in the field. The incidence of conidiospores was highest at the booting stage, which is the sensitive infective stage, and conidiospores are the main sources of infection.

Hegde et al. (2000) reported the influence of weather parameters on the incidence of false smut of rice on its seeds. The correlation studies indicated that weather parameters during 50% flowering had a significant effect on false smut disease development in rice. Low maximum temperature (<31 °C), low rainfall (<5 mm), high minimum temperature (19 °C) and high relative humidity (>90%) during flowering were favourable for disease transmission and development. Although seed transmission of *U. virens* is relatively poor than the wind-borne conidial transmission and infection, it is important to understand the favourable conditions responsible for such air-borne transmission and infection in rice plant and seeds in particular. In this regard, Devi and Singh (2007) monitored air-borne *U. virens* (syn. *C. oryzae sativae*) spores over the rice field in Manipur, India, by using the Rotorod air sampler. Symptom on the host was visible only after panicle emergence at milk stage of the individual grain. They observed a rapid development of infection when the crop passed through growth stage 10–11, i.e. ripening to harvest stage. Smut incidence was also correlated with spore concentration in the air, growth phases of the crop and meteorological parameters. The highest spore catches (41.661/m^3) coincided with the maximum of 29 °C and minimum of 13 °C and 89% relative humidity. During next crop season, the spores started to appear in the air and the highest spore catch (40.118/m^3) was recorded with maximum of 27 °C and minimum of 13 °C temperatures and 95% optimum relative humidity. During both the season, there was nil rainfall. Therefore, such environmental conditions are crucial for successful infection and transmission of false smut pathogen under field conditions.

26.6.6.1 Infection Cycle of *U. virens*

To study the infection process, Tanaka et al. (2011) developed a simple procedure for the transformation of the pathogen using electroporation of intact conidial cells and ascertained infection process of the pathogen. They constructed a transformation vector pCB1004eGFP with a green fluorescent protein (eGFP) gene under a constitutive promoter of the glyceraldehyde-3-phosphate dehydrogenase gene of *Cochliobolus heterostrophus*. When a linearized vector was applied, eGFP-expressing transformants were successfully acquired. An inoculation test in rice plants at booting stage expressed the eGFP gene, which was integrated with the pathogen gene and was able to form rice false smut balls (Figs. 26.14 and 26.15)

26.6.7 Sheath Blight

This is major disease occurring in most of the Asian countries including India. The pathogen *Rhizoctonia solani* is a polyphagus fungus, which attacks different species of rice and grasses as weed hosts in rice fields. The infected leaf sheath (mostly lower portion) acts as a primary source of inoculums under favourable conditions. Infection spread into all parts of seeds. Severely infected leaves show bands and

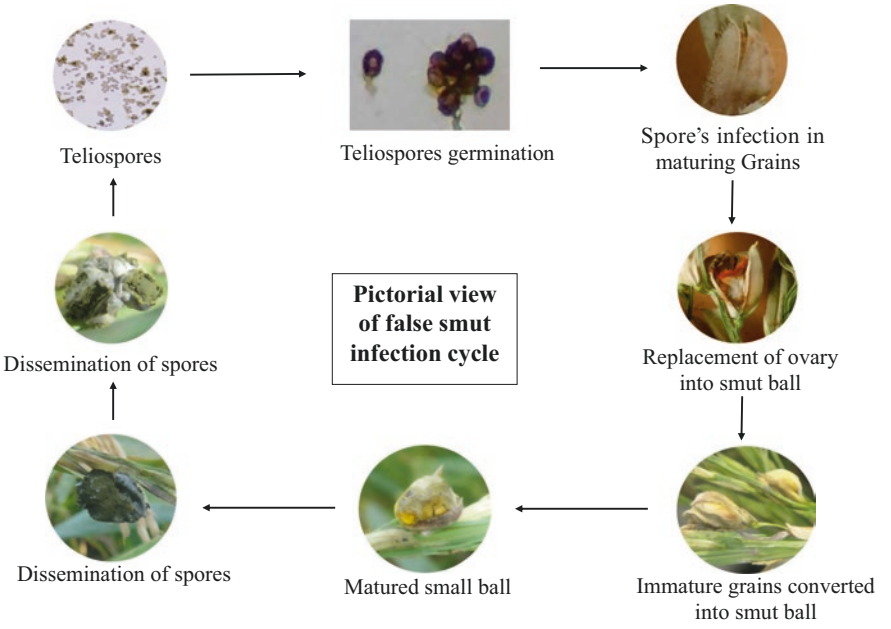

Fig. 26.14 Infection cycle of false smut in rice

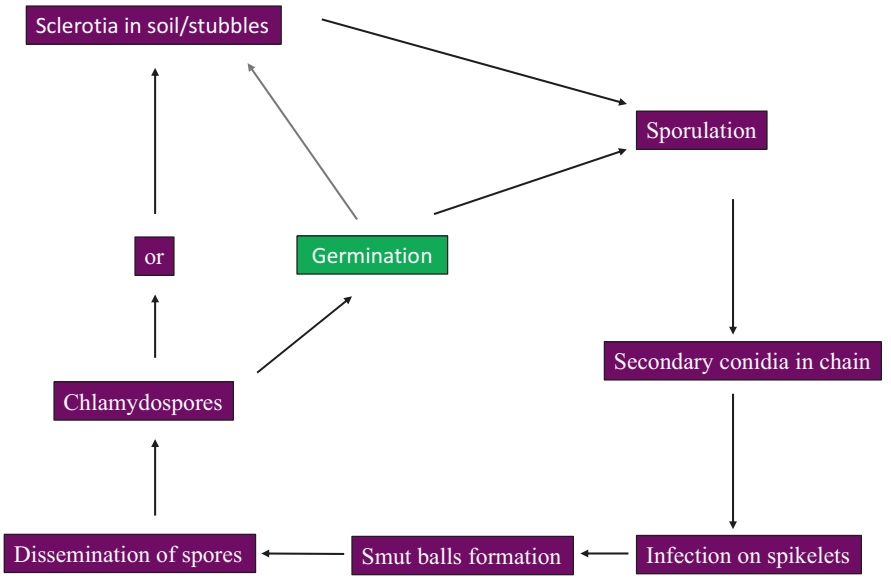

Fig. 26.15 Schematic representation of transmission cycle of *U. virens* from seed/soil to seeds

hence it also produces banded blight-like symptoms as in the case of other millets and maize. Partially systemic transmission takes place from hard sclerotia formed between leaf sheaths. Infection reaches up to panicles and the active inoculum from such infection becomes seed-borne in rice for subsequent cropping period.

26.6.8 Sheath Rot

Earlier, this was a minor disease but in most of the humid regions, significant loss in yield and quality of rice has been reported. Muralidharan and Venkata (1980) recorded up to 80% loss in yield, although Chakravarty and Biswas (1978) recorded 26% yield loss. The pathogen *Sarocladium oryzae* infects almost all parts of plants, most preferably the upper portion of leaf sheath which encloses young panicles. Severely infected panicles are sterile or form shrivelled grains with brown discolouration based on the severity of infection. Regarding transmission, Hsieh et al. (1980) could not record the evidences for seed transmission; it was proved by Chuke in the year 1983. Milagrosa (1987) recorded transmission of the pathogen from seeds to adult plants (cv. IR 36, CR 333-1-Z). The systemic nature of pathogen was proved by isolating the fungus from all parts of infected plants.

26.7 Mechanisms and Mode of Entry for Infection of Seed-Borne Bacteria

Transmission and infection of seed-borne bacteria are different from seed-borne fungi and viruses. Since entry of the bacteria is normally passive into tissues of target plants, movement is mostly associated with flagella and their arrangement on pathogenic bacteria. For example, in peritrichous flagellation, numerous flagella are present all over sides of bacterial cells (*Erwinia* spp); in monotrichous, single flagellum is present as in the case of *Xanthomonas* spp. (*Xanthomonas campestris* pv. *malvacearum*); and in amphitrichous, single flagellum or multiple flagella are located on both ends of the bacterial cell as in the case of *Pseudomonas* spp. In all these species, the fimbriae help in attachment of the bacteria to drive nutrients from the substrates which are specific to some sugars (mannose). The infective bacterial cells migrate and accumulate in most optimum concentration of chemical gradient. However, the chemicals may be attractants or repellents (phenomenon known as chemotaxis). Since pathogenic bacteria possess memory for temporal gradient sensing system and the memory is lost for several seconds, it can compare the concentration of environment over distance. Based on the necessity and nature of chemical gradient, the cells move towards the substrate. It was reported that the frequency of tumbling will be more towards toxic gradient or environment. After gaining entry, bacteria initiate infection process.

The mechanisms of transmission of seed-borne bacteria vary with different species of seed pathogenic bacteria. Stomata openings help in entry of bacteria (*Xanthomonas campestris* pv. *phaseoli*). Bacteria enter through film of water that extend outside the stomatal aperture into the roots. However, stomatal morphology

determines the success of transmission. For example, in the case of citrus canker, conformation of the cuticle around the stomata either prevents or allows the passage of water droplets with the bacteria for transmission. Natural openings like nectarthodes, hydathodes and trichomes also help in transmission of bacteria. Occurrence of nectarines on saucer-shaped tissues between the point of emergence of style and stamens helps in entry of *Erwinia amylovora* which enter through such nectarines of apple and pear flowers. A droplet of nectar comes out from stomata like nectarthodes and beneath such nectarthodes, bacteria colonize abundantly within 24–48 h of entry. Similarly, hydathodes also play a role in transmission of seed-borne bacteria. These are the structures through which water is discharged from the interior of leaves, directly from bundles of terminal tracheids. The pathogenic bacteria like *X. campestris* and *E. amylovora* specifically enter through such hydathodes. The BLB pathogen (*Xanthomonas oryzae* pv. *oryzae*) can accumulate in water from hydathodes and is drawn into epithem and tracheids. In some cases, the pathogenic bacteria enter through trichomes (*Corynebacterium michiganense* of tomatoes) to cause cankerous symptoms. Apart from these natural openings, seed-borne bacteria gain direct entry also. For example, the wounds may be natural or man-made due to agricultural operations or insect pest facilitates the entry of bacteria (*Xanthomonas stewartii*). Bacteria overwinter in intestinal tracts of insects (flea beetle) like *Chaetocnema pulicaria* and *C. denticulata*. Plants get infected when the beetles start to feed in spring season on maize plants.

Similar to other seed-borne pathogens, seed-borne bacteria also enter either systemically or non-systemically. Systemic transmission occurs through vascular elements (*X. campestris* pv. *brassicae*), first survival on infected seeds and transmission to seedlings and lateral entry and infection (*Pseudomonas avenae*). Bacteria are also transmitted through fruit stalks. In the case of *Xanthomonas campestris* pv. *pruni* and *E. stewartii*, they enter into vascular system of leaves and shoots and enter into fruit stalks and seeds. In some cases, infected seeds act as a symptomless carrier and facilitate infection. For example, *Pseudomonas syringe* pv. *lachrymans* survives in vascular system of *Cucumis sativus* and move into fruits and, finally, into seeds. In the case of halo blight of bean, the pathogenic bacterium *X. campestris* pv. *phaseoli* enters into vascular system without symptoms but it enters through funiculus and subsequently to raphe and then seed coat of beans. The peduncle, siliqua and funiculus are also playing an important role in transmission of seed-borne bacteria (e.g. *Xanthomonas campestris* pv. *campestris*). Seed-borne bacteria infect through sepals and then peduncle and kill flowers with shrivelling of young seeds and from there it migrates to seeds via funiculus, then micropyle and into the seed coat.

Some of the seed-borne bacteria transmit through the seed coat; like black arm of cotton (*X. campestris* pv. *malvacearum*), bacteria enter through the basal end at chalaza and into seeds. In case of seed-borne bacteria of tomatoes (*Corynebacterium michiganensis* subsp. *michiganensis*), the infection begins at the chalazal end and continues into the innermost cells of the seed coat. In castor, the seed-borne bacteria (*Xanthomonas campestris* pv. *ricini*) enter through the capsule, through the pericarp and into the seed integuments. Bacteria also enter from the external seed coat (lesions on pods) and enter into underlying seeds (bean). In the case of rice,

Pseudomonas glumae colonizes the surface of basal portion of the lodicules and inner surface of the lemma and from there, it enters the outer epidermis and seeds. Therefore, the mode of entry and transmission of seed-borne bacteria differ with crops and diseases. In soybeans, the bacterium *Pseudomonas syringae* pv. *glycinea* can be seed-borne and overwinters in the soil and on soybean residue. The infected seed can lead to seed decay. If conditions are favourable early in the season, infection can occur on cotyledons and can cause seedlings to be stunted or killed. Leaf infection usually occurs early to midseason under moist conditions. Bacterial blight spreads when bacteria are disseminated by wind, rain splash and by farm equipment passing through the crop. Seeds may be colonized, becoming shrivelled with sunken and discoloured lesions. Infected soybean residue or seed-borne inoculum spreads to plants by wind-driven rains. Early infections may appear severe, especially in wet weather, but hot dry conditions restrict the disease development. Bacterial blight of Indian bean and cluster bean incited by *Xanthomonas campestris* pv. *cyamopsidis* is also seed-borne and this is a major disease in Rajasthan, India. We can observe yellow oozing from infected plants. The bacteria enter through opening of the attachment or hilum or stomata, and systemic infection makes it deep-seated in embryonic tissues. Keeping in the view of vast information on these aspects, a common and major seed-borne disease, i.e. bacterial blight, of rice is described as follows.

26.7.1 Bacterial Leaf Blight (BLB) of Rice: Mechanisms of Transmission and Infection of *Xanthomonas oryzae* pv. *oryzae* in Rice Seeds

Bacterial leaf blight of rice was first recorded by Japanese in the year 1884 (Tagami and Mizukami 1962). It is well known that the 'Kresek phase' which is a highly destructive form of this disease is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Tender leaves of the infected plant turn pale yellow and wilt during the seedling to the early tillering stage, resulting in severe loss in crop growth. Mew (1987) recorded it as a vascular disease resulting in a systemic infection. Bokura in 1911 named the causal organism as *Bacillus oryzae* Hori and Bokura, later on named as *Xanthomonas campestris* pv. *oryzae* Dye (Mew 1987) and presently as *Xanthomonas oryzae* pv. *oryzae* (Swings et al. 1990). Niño-Liu et al. (2006) reported that in bacterial blight and bacterial leaf streak pathogens of rice (*Oryza sativa*), elucidation of mechanisms of pathogenesis revealed that there are 29 major genes for resistance to bacterial blight, but so far only a few quantitative resistance loci for bacterial leaf streak have been reported. Members of the AvrBs3/PthA family of transcription activator-like effectors play a major role in the virulence and pathogenesis of *X. oryzae* pv. *oryzae* and possibly *X. oryzae* pv. *oryzicola* in rice. Over 30 resistance genes, termed *Xa1* to *Xa33*, have been identified in rice plants, which are counteracting with virulence of *Xoo* infection (Hugo 2013).

26.7.1.1 Seed-Borne Infection

Kiryu et al. (1954) concluded that abundant inoculum results in higher percentage of infection. Horino (1984) and Mew et al. (1984) reported that the successful infection of a rice plant by *Xoo* involves the movement of the bacterium towards the host, contact between the two, penetration of the host by the bacterium and proliferation of the bacterium inside the host immediately following entrance. *Xoo* enters through hydathodes or wounds on the rice leaves. It was proved that the infection seems more successful and significant in the case of passive entry of *Xoo* through wound sites than natural openings. However, new wounds are more conducive to infection than old wounds.

26.7.1.2 Seed Transmission of *Xoo*

In general, any pathogenic bacteria multiply in intercellular spaces (the apoplast) after entering through gas or water pores (stomata and hydathodes, respectively) or gain access via wounds (Jones and Dangl 2006). The seed-borne inoculum acts as a primary source of transmission into healthy seedlings and adult plants. However, irrigation water is also considered to contribute to the spread of this disease over large areas of cultivated land, as it carries the bacterial ooze that drops into rice field. Tagami et al. (1963) had different opinions on the role of water as a primary mode of transmission, perhaps due to short period of pathogen survival (only 15 days) in field water. Unnamalai et al. (1988) reported *Xoo* as seed-borne, although the extent to which it is transmitted through the seed was not clear. Hugo (2013) found that the rice fields are flooded during most of the growing season. Therefore, *Xoo* may easily spread among crops, i.e. the bacteria travel through the water from infected plants to the roots and leaves of neighbouring rice plants. Wind and water may also help spread *Xoo* bacterium to other crops and rice. Different documents revealed various mechanisms including quorum sensing and biofilm formation during symptom expression and test pathogen (*Xoo*). In non-growing seasons, *Xoo* may survive in rice seeds, straw, other living hosts, water or, for brief periods, soil in addition to infection in other collateral hosts like cutgrass (*Leersia oryzoides*), common grasses and weeds. However, the seed-borne transmission of *Xoo* either in the nursery or in the field is important to formulate management strategies.

26.8 Mechanisms of Transmission and Infection of Other Seed-Borne Pathogens in Millets

Apart from wheat and rice, millets are also grown in semi-arid and arid regions of the country. These crops are also adversely affected by smuts and downy mildew under different cropping seasons and areas. Sorghum (*Sorghum vulgare* Pers.) is grown in warmer conditions with very low rainfall. This dryland major millet is grown for seed and grain purposes not only in India but also in other parts of the world. Out of different diseases, smuts are major seed-borne diseases. These are

covered smut (*Sphacelotheca sorghi* (Link) Cliut), loose smut (*Sphacelotheca cruenta* (Kuhn)), long smut (*Tolyposporium ehrenbergii* (Kuhn) Pat) and head smut (*Sphacelotheca reiliana* (Kuhn) Cliut). Out of these, the covered smut also known as kernel smut is most serious disease in southern states. Most of the grains infected by loose smut pathogen are converted into sori in place of normal grains. This sorus (pl. sori) is covered by intact tough membrane which ruptures to release the teliospores as primary inoculum. A long sorus consists of central hard columella extending from the base to the apex. The dark powdery mass of spores is dispersed after rupturing of membrane and germinates in free water to form four-celled promycelium with sporidia and the viability of spores could be retained up to 10–12 years. Hence, transmission of pathogen is from infected sori to soil or seeds. It has been documented that the germinated spores gain entry through the radicle or mesophyll region immediately after germination of sorghum seeds. Infective hyphae colonizes within the meristematic tissues and keep pace with the growth of the plants and, therefore, become systemic in nature of infection and transmission.

It is reported that the two compatible monosporidial lines are required to become infective; otherwise, single monosporidial lines may not be effective for infection. Low temperature about 25 °C is conducive for the pathogen to have at least 50% infection in germinating seeds (Kulkarni 1922); otherwise, infection will be poor at higher temperature. Reed and Faris (1924) viewed that soil moisture in addition to type of soil (sandy) and temperature play a vital role in smut infection in sorghum seeds.

In the case of loose smut, the infected ear heads are loose which emerged from unhealthy and thinner stalks formed from the infected seeds. Since majority of the spikelets are infected, sori are developed on all floral parts. The pathogen's infection is almost similar to covered smut but the chlamydospores are viable up to 4 years in infected seeds or debris. Transmission is reported from infected seeds to soil which ultimately infects germinating seeds and the pathogen progresses into seedlings. However, primary infection is reported only from seeds. The rest of the infection process and symptom expression are similar to covered smut of sorghum. Another smut, *Tolyposporium ehrenbergii*, infects only few grains of sorghum which is sporadic in nature. Primary infection starts from air-borne sporidia.

Apart from smuts, the downy mildew is the second major seed- and soil-borne biotrophic pathogen in millets. Symptoms can be recognized on the plants particularly on leaves. Lateral tillers with stunted growth and shredding are the common symptoms observed when the inoculums are present in soil and seeds. The transformation of the ear into green head with leaflike structure is a visible characteristic symptom of this disease (Fig. 26.16). The pathogen *Sclerospora graminicola* is systemic in nature and readily colonizes on almost all parts of the plants but usually confined to parenchymatous tissues. Although downy mildew is systemic disease, infective hyphae or any form of inoculums is not deep-seated but abundant oospores (dormant spores) are adhered on the seed surface during harvest and threshing and therefore, they act as a primary source of inoculum for seedling infection after germination along with seeds. The germ tube penetrates directly on the cells of roots, coleorhizae and coleoptiles of

Fig. 26.16 Systemic transmission of *Sclerospora graminicola* and symptom expression as green ear head (Pear millet: Field photo from Ghaziabad, NCR, Delhi)

tender seedlings and therefore becomes systemic in nature for onward progression and symptom expression. The mode of transmission and infection of downy mildew pathogen is almost similar in the case of other millets.

26.9 Transmission and Infection of Seed-Borne (SB) Viruses

Most of the plant viruses are transmitted by insect vectors, mechanical or sap transmission, etc. However, few viruses which are systemic to plant system are carried by seeds for subsequent infection. Basu and Giri (1992) reported that about 100 viruses are transmitted through seeds. Some of the best examples are *Alfalfa mosaic virus*, *Barley stripe mosaic virus* (BSMV), *Bean common mosaic virus* (BCMV), *Bean yellow mosaic virus* (BYMV), mosaics in pulses and cucumber, *Pea seed-borne mosaic virus*, *Soybean mosaic virus*, *Tomato black ring virus*, etc. The seed-borne viruses are located in different parts of seeds such as the embryo (wheat and barley stripe, *Bean common mosaic virus*, *Cowpea mosaic virus*, *Tobacco ringspot virus* (TRSV) and *Cherry necrotic ringspot virus*), endosperm (wheat and barley stripe, *Bean common mosaic virus*, BSMV, CMV, TRS) and seed coat (CMV, *Tobacco mosaic virus*, BCMV and *Tomato spotted wilt virus*). Different factors, viz. virus or virus strains, host species, cultivar and environmental conditions, determine the success of transmission. In crop plants, approximately 20% of viruses are transmitted from generation to generation in the seed (Mink 1993). Seed transmission of plant viruses results from the three-way interplay of the genetic components of the virus, the host material and its progeny (Maule and Wang 1996). Seeds offer a highly effective barrier to passage of most viruses from one generation to the next, and perpetuation of most of the highly destructive viruses is still dependent on repeated infection of successive generation of plants through the agency of natural vectors. Bennett (1969) reported that the most plant viruses' relationship to their host plants is the high degree of protection possessed by embryos of seeds against invasion by viruses that affect the mother plant. It was further emphasized that the ability of virus to infect male and female gametophytes is one of the

determining factors involved in seed transmission of viruses in majority of cases. But an appreciable number of viruses has been found to pass from one generation to the next through the medium of the seed. Ali and Randles (1998) reported the effect of the South Australian pathotype 4 isolate (S6) of *Pea seed-borne mosaic virus* (PSbMV-S6) on the yield components of peas. This virus not only severely affects seed yield but changes plant habit and time of maturation in field crops. Seed yield was reduced by 82% and the virus (PSbMV-S6) was transmitted to 31% of seedlings. Ali and Kobayashi (2010) detected seed transmission of *Cucumber mosaic virus* (CMV) in pepper for the first time. Total RNA was extracted from pepper seeds and analysed by reverse transcription-polymerase chain reaction (RT-PCR) using CMV subgroup IA specific primers. Analysis of individual whole seeds showed seed-borne infection of CMV in pepper ranging from 95% to 100%. Further CMV was detected in both the seed coat and embryo by seed growth tests. Seed coat infection of CMV ranged from 53% to 83% while that of the embryo ranged from 10% to 46%. Coutts et al. (2009) reported effects of seed-borne inoculum on virus spread, yield losses and seed infection in the *Pea seed-borne mosaic virus*-field pea patho-system. Yield losses of 18–25% with less seed weight and seed number were recorded when final incidence reached 97–98% in plots sown with 6.5–8% infected seeds. Plants with symptoms and incidence increased with the amount of primary inoculum present in seeds. Cereal crops are relatively free from seed-borne virus diseases as compared to pulses, fruits and vegetable crops, perhaps due to diversified weather conditions in different agroclimatic regions in India.

In major cereals like rice, virus diseases are not important as compared to fungal and bacterial diseases except few insect-transmitted virus diseases. Hibino (1996) reported 15 viruses from rice (black-streaked dwarf, rice bunchy stunt, rice dwarf, rice gall dwarf, rice giallume, rice grassy stunt, rice hoja blanca, rice necrosis mosaic, rice ragged stunt, rice stripe necrosis, rice stripe, rice transitory yellowing, rice tungro bacilliform, rice tungro spherical and rice yellow mottle viruses). However, Abo and Sy (1997) documented 30 viruses; however only 25 are of any direct economic impact to rice production. The majority of these viruses are prevalent in Asia and the Americas, whereas only five viruses, namely, *Rice stripe necrosis virus* (RSNV), rice crinkle disease, *Maize streak virus* (MSV), *African cereal streak virus* and *Rice yellow mottle virus* (RYMV), infect rice plants in nature in Africa and its neighbouring islands. Most viruses that are seed-borne can be carried along with the seeds. Ou (1985) reported seed-borne viruses, namely, *Rice wrinkled stunt virus* and *Rice witches broom virus*. Some seedlings could be infected by these viruses from the nursery and carried to fields during transplanting. *Rice yellow mottle virus* was first noticed in Kenya, East Africa, in 1966. It is believed to have come to the foreground with the introductions of exotic rice (*Oryza sativa* L.) from Asia into the continent (Abo et al. 1997). However, Awoderu (1991) reported that the possibility of the RYMV being transmitted either by the beetle vector or through being carried on the seed. Konate et al. (2001) detected two pathogroups of RYMV by using immunological method (ELISA). Most of the susceptible genotypes responded with positive reactions. They confirmed the presence of virus up to 100% in all parts of seeds including the glumella, endosperm and embryo. However, the

infectivity of virus was declined during the process of seed formation and inactivated at the time of seed maturation and desiccation. Fauquet and Thouvenel (1977) studied sap transmission of RYMV causing a mosaic disease on IR 8 and Jaya irrigated rice in the Ivory Coast. It was indicated from serological tests as a strain of RYMV (RPP 54, 3297) with isometric (28 nm diam.) sizes.

26.9.1 Mechanisms Involved in Viral Infection and Transmission

Carroll (1981) reported that the process of virus seed transmission is influenced by the environment in addition to a consequence of specific interactions between the virus and the combined physiology of the host plant. Successful transmission of seed-borne viruses depends on the vulnerability of seeds. Mandahar (1981) documented that transmission of viruses is achieved either by direct invasion of the embryo via ovule or by indirect invasion of the embryo, mediated by infected gametes. In some cases, like *Barely stripe mosaic virus* (BSTV), both processes can happen simultaneously. Wang and Maule (1992) found that the seed-borne mosaic virus of pea (PSbMV) invaded pea embryo directly during early development and multiplies within embryonic tissues, which resulted into seed transmission. Roberts et al. (2003) reported that seed transmission of *Pea seed-borne mosaic virus* (PSbMV) depends upon symplastic transport of the virus from infected maternal cells to the embryo. They studied the ultrastructure of the tissues and cells around the micropyle of young developing seeds and compared transmitted and non-transmitted virus isolates. The presence of cylindrical inclusions on the testa-endosperm boundary wall, together with immuno-gold labelling for virus-specific products on the wall and in the endosperm, indicated that symplastic connections existed at this interface. Close examination of the endosperm-suspensor boundary at the base of the suspensor revealed discontinuities in the suspensor sheath wall as pore-like structures, in which the virus might pass through en route to the embryo.

Invasion of the embryo occurred via the suspensor (a transient structure in embryo development which is meant for conduct and nutritional support of the growing embryo). Kawashima and Goldberg (2010) defined that the suspensor is a terminally differentiated embryonic region that connects the embryo to surrounding tissues during early seed development. Most seed-bearing plant embryos contain suspensor regions, which occur in a wide variety of sizes and shapes, and suspensor-like structures are present in the embryos of some lower land plants. Recent works on molecular biology with seed transmission including novel genomics approaches have provided insights into the function of the suspensor and the DNA sequences that control suspensor-specific gene expression. The genetic basis for this interaction was recorded in the case of barely infected by *Barley stripe mosaic virus* (BSTV). A single recessive gene was involved in regulation of seed transmission of this particular virus (Carroll et al. 1979). The molecular mechanisms controlling embryo basal cell lineage specification and suspensor differentiation events are also beginning to illuminate. Similarly, there are many numbers of seed-borne viruses which are transmitted through true seeds and planting materials.

26.10 Mechanisms of Transmission and Infection by Seed-Borne Nematodes in Rice and Wheat

Plant parasitic nematodes are the main constraints in crop production and productivity. Handoo (1998) reported an estimated yield loss to the tune of $80 billion and the recent estimate by Singh et al. (2015) projects yield loss of 12.3% ($157 billion dollars) worldwide and $40.3 million from India. About 4100 species of nematodes have been identified to infest crop plants (Decraemer and Hunt 2006).

Nematodes are highly subterranean and hence they infest and contaminate mainly the soil-borne seed materials such as peanut, potato and root stocks of several plants. However, very few nematodes such as *Aphelenchoides besseyi* in rice and *Anguina tritici* in wheat can infest and spread through seeds developed on aerial parts. This ability to attack aerial parts made these nematodes as of quarantine importance and has been highly cited in quarantine laws of several countries (Kahn 1982). Plant parasitic nematodes are microscopic in nature, simple in body organization, have ability to tolerate wide range of environmental stresses, have short life cycle, have high fecundity, have ability to overwinter on plant residues for very long time, coupled with farmer's ignorance and absence of clear visible symptom on plants. The mechanisms of transmission and mode of infection of nematodes associated with rice and wheat seeds are described as follows.

26.10.1 White Tip Disease in Rice

White tip disease incited by *Aphelenchoides besseyi* (Christie 1942) was recorded in an epidemic form from central India during 1934 (Dastur 1936), subsequently by Christie (1942) from Assam and Odisha. This particular nematode pest was also reported from states of Tamil Nadu, Karnataka, Maharashtra, West Bengal, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Odisha, Jharkhand and Himachal Pradesh. However major distribution and incidence concentrated around eastern region of India. Hoshino and Togashi (1999) and Bridge and Starr (2007) reported yield loss to the tune of 50% by inducing stunting and sterility. In addition to rice, it also infests strawberry, tuberose, weeds and other crops of Poaceae family. The nematode is characterized by squarish metacarpus, mucros with three points at tail tip, small stylet with knob and males with rose thorn-shape spicules without bursa.

26.10.1.1 Symptoms

Whit tip symptoms are observed on the top of leaves with a measure of one cm to one-third of the total length. The emerging leaf becomes whitish (Fig 26.17a) or whip-like followed by necrosis. Such symptoms are confused with albinos (Fig 26.17c) in seedling stage. Twisting and wrinkling of developing leaves including flag leaf are also noticed (Fig 26.17b). Severity of infestation causes stunting, shorter panicle, delayed maturity, less grain, shrunken grain or chaffiness.

Fig. 26.17 (**a**) White tip symptoms on rice leaves, (**b**) Twisting and wrinkling and (**c**) complete chlorosis of leaves infested with *Aphelenchoides besseyi*

26.10.1.2 Mechanisms of Survival and Transmission

The pathogenic populations of *A. besseyi* survive in anhydrobiotic stage (cottony wool) under the seed glumes, which can harbour 5–6 nematodes per seed. When seeds are sown in nursery beds, the seeds imbibe water to germinate and the nematodes present inside the seeds also absorb water for rehydration and revival. The nematodes start feeding on the growing apical bud (meristem). They generally feed from inside the leaf whorl during nursery and early tillering stages, thus escaping the external harsh environment. Within few days the juveniles moult into adults and start to reproduce sexually (amphimixis). In rare cases the reproduction takes place parthenogenetically. The eggs are laid in thin film of water present inside the whorls. The egg to egg cycle completes within a short span of 8–12 days (Hoshino and Togashi 2000; Bridge and Starr 2007); as a result, the nematode population increases dramatically during crop growth. When the plant reaches the reproductive stage, the nematodes migrate to the florets by harnessing the thin film of water available via dew. After reaching the reproductive part, they start feeding the internal content of florets and eventually settle inside the developing seeds. Then the nematode desiccates along with the drying seed and the nematode can remain viable inside the seed for 3 years. This nematode species is generally thermophilic and its optimum temperature requirement varies from 23 to 30 °C.

26.10.2 Ear Cockle Disease of Wheat

Anguina tritici is the first plant parasitic nematode reported in 1743 by John Turberville Needham, a catholic clergyman. He observed white fibrous substance in blackened wheat grains which got separated in a drop of water. It was first named as *Vibrio tritici* by Steinbuch (1799) and subsequently, Filipjev (1936) gave the name

as *Anguina tritici*. This nematode also infests barley, oats and rye in addition to wheat. In India, ear cockle was first reported by Hutchinson (1917) and its widespread distribution in all wheat belts was reported by Koshy and Swarup (1971). In general, incidence level of the disease varies from as low as 1%. However, in an individual field, it may be as high as 50%. Mustafa (2009) observed that the baking quality is reduced because of reduced gluten content when the galls are present at the rate of >5% in a grain lot. Females of *A. tritici* are long and obese and form close spiral/S-shaped/ventrally arcuate and have broader middle portion and tapering ends. The oesophagus is prominent with a muscular medium bulb. The ovary is well developed with two or more flexures at the anterior end. The male copulatory organs (spicules) are broad and short.

26.10.2.1 Mechanisms of Survival and Transmission

The second stage juveniles (J2) are present in galled seeds of wheat in an anhydrobiotic stage. When such galled seeds are sown, the juveniles inside seeds get rehydrated from available soil moisture and emerge out and migrate towards the growing epicotyl and start feeding on the growing tip. The nematodes feed on growing leaf buds as ectoparasites all through the vegetative stage of the crop. During this period the nematodes are carried mechanically along with growing plants. Upon reaching reproductive stage, i.e. initiation of floral primordia (~70 days), it penetrates and enters into developing seed and starts feeding on its internal content. In the early stage, externally the seed seems to be green and healthy (green galls), where the J2 undergoes three successive moultings to become J3, J4 and adult (~90 days). The number of adults present in the green galls varies from 16 adults (Leukel 1924) to 85 adults (Marcinowski 1909) and to the maximum of 283 adults (Midha and Swarup 1972). Mating takes place inside the galls and the adults die after oviposition (~110 days). The eggs develop into JI and first moult takes place inside the egg and J2 hatches out of the egg. As each female has the ability to lay more than 100 eggs, the final J2 population of a single gall reaches from 3000 to 20,000 (Fig. 26.18a). Later the J2 undergoes slow anhydrobiosis inside the gall along with maturing plant, where there will be no visible sign of life or cell metabolic activity. Through anhydrobiosis, the nematode has the ability to survive 25–28 years inside the galls (Needham 1743; Fielding 1951). Midha and Swarup (1972) reported that emergence of juveniles is favoured by an optimum soil temperature of 15 °C, 20% soil moisture at 2 cm depth.

The nematode sometimes in association with bacterium, *Clavibacter tritici*, causes yellow slimy mass on the ear head. These slimy mass oozes out, when the humidity rises. This slime after drying up becomes brittle and as a consequence, it hampers the milling and post-processing storage of wheat. The bacterium is found to be present invariably along with the nematode larva inside the galls and the condition is called yellow ear rot or Tundu disease. Severity of the disease causes 100% crop failure (Gupta and Swarup 1972).

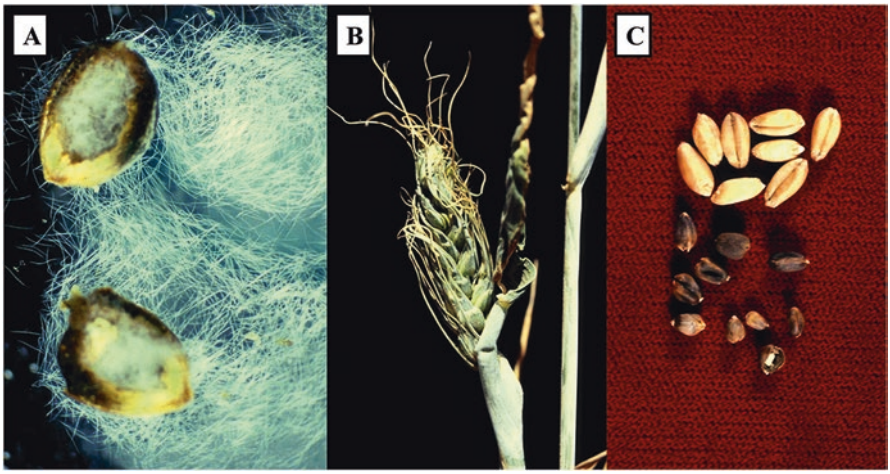

Fig. 26.18 (**a**) Teased galls showing thousands of infective juveniles. (**b**) Infested ear head. (**c**) Healthy seeds above and infested galls below *Image: Michael McClure, University of Arizona, Bugwood.org*

26.10.2.2 Symptoms

The first symptom appears on 20–25th day as enlargement of the base of the stem near the soil followed by crinkling, rolling and twisting of leaves on 30–35th day (Fig 26.18b). All these early symptoms disappear at later stages. When the infestation is severe, the plant will be stunted and die prematurely. Otherwise, they grow fast with profuse tillering and reach reproductive stage 30–40 days earlier as compared to healthy plants. However, the ear heads remain short with spreading glumes, bearing nematode in green galls. This later develops into hard, shrivelled brown to black galls (Fig 26.18c) with thousands of nematodes inside. The galls are non-toxic, when fed to cattle. The infection may be partial or complete in an ear head and is known as purples or pepper corns (Gupta and Swarup 1968). Seed galls upon small jerk fall to the ground as they are loosely attached in the ear heads (Swarup and Sosa-Moss 1990) (Fig. 26.19).

26.11 Conclusion

Although seed-borne pathogens including viruses and nematodes result relatively in less yield loss than foliar fungal and bacterial pathogens of adult plants, few examples of seed-borne pathogens discussed in this chapter will lead to formulate suitable management strategies. Most of the seed-borne pathogens are subjected to quantitative measurements than qualitative analysis and, therefore, become difficult to eradicate them. The titre or concentrations of seed-borne virus may vary with different compartments of seeds. Since seed-borne fungal and viral pathogens are located in the endosperm and embryo of cereals, management practices having

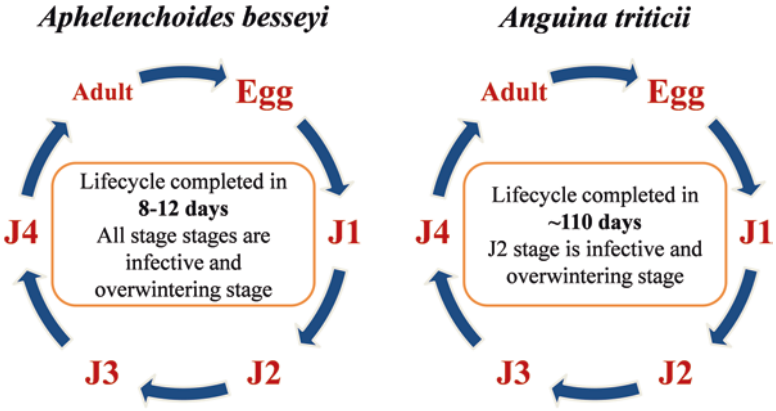

Fig. 26.19 Survival, infection process and life cycle of *Anguina tritici* and *Aphelenchoides besseyi* in wheat and rice seeds, respectively

more than one component are not even successful to eliminate such pathogens from seeds. Fungicides as seed treatment proved to be a success but toxicity on mammals still stands for global debate. Some of the fungicides became ineffective possibly because of development of resistance over the period and lack of understanding about location and transmission of target pathogens. Therefore, besides all other integrated measures, it is imperative to develop durable resistant varieties preferably through tissue culture and transgenic approaches to counteract with seed-borne pathogens.

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Part X

Miscellaneous

Seed Health Testing and Seed Certification

27

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Abstract

Seeds are the basic input for crop production. Infected or contaminated seed is the primary source of inoculum for a large number of destructive diseases of important food, fodder and fibre crops. In this context, seed health tests have become a major component towards sustainable crop production and crop productivity. Apart from seed health testing, the seed certification for a crop comprises legal norms to be qualified for ensuring genetic identity, physical purity, germinability and freedom from seed-transmitted pathogens and weeds. Mainly different types of conventional methods followed by molecular methods are employed for seed health testing. Conventional methods include visual inspection of seeds, washing test, seed soak method and incubation methods, while serological assay and nucleic acid-based techniques are the most accurate and least time-consuming which comes under molecular method of seed health testing. The conventional methods are mostly based on interpretation of visual symptoms, culturing and laboratory identification. Thus, it is almost difficult to interpret results with hidden symptoms. However, among all techniques available, nucleic acid-based techniques are the most accurate and least time-consuming.

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27.1 Introduction

Seeds are the basic input for crop production. About 90% of the world's food crops are sown using seeds. The major world food crops are rice, wheat, maize, barley, beans, millet, peanut, pulses, sorghum and sugar beet. These crops are attacked by a large number of pathogens, a majority of which are seed-borne in nature. Crop diseases in general and seed-borne diseases in particular are of great economic significance, as they are responsible for causing heavy economic losses. It is a well-established fact that seeds are both the victim and the vehicle of the extensive and complex microflora. There may be hardly any cultivated crop, where at least one seed-transmitted disease is not known. Some of the most important plant pathogens are seed-borne and seed-transmitted. The various disease-causing microorganisms, i.e. fungi, bacteria, viruses and even nematodes, are carried with the seeds/planting materials. However, the fungal plant pathogens are the most predominant ones. Infected or contaminated seed is the primary source of inoculum for a large number of destructive diseases of important food, fodder and fibre crops (Neergaard 1977).

In this context, seed health tests have become a major component towards sustainable crop production and crop productivity. Seed health refers to the presence or absence of disease-causing organisms which may be fungi, bacteria, viruses and animal pests such as eelworms and insects and also abiotic stresses involved to cause deterioration in seed quality and quantity. In seed health testing, we deal with the detection and identification of seed-borne microorganisms. The objective of seed health testing is to determine the health status of a seed sample so that the quality of different seed lots can be judged. It may elucidate seedling evaluation and causes of poor germination or field establishment and thus supplement germination testing. Thus, it is imperative to analyse the seeds for associated pathogen. It also checks the development of a disease which may become epidemic under favourable environmental conditions. Sometimes, even saprophytes like *Aspergillus* spp. and *Penicillium* spp. are not taken as a serious concern, but they may be responsible for the loss of viability of seeds during storage.

Apart from affecting the yield, many seed-borne pathogens reduce seed germination and vigour of seedlings and induce qualitative changes in the physicochemical properties of seeds, such as colour, odour, oil content, iodine and saponification value, refractive index and protein content, thereby affecting their commercial value (Agarwal and Sinclair 1997).

27.2 Detection Methods of Seed-Borne Pathogens

The selection of a detection method depends upon the purpose of the test, i.e. whether the seeds are to be tested for seed certification, seed treatment, quarantine, etc. If it is for quarantine purposes, then it is imperative to use highly sensitive methods, which can detect even traces of inoculum. The various techniques including conventional and modern methods that are employed for seed health testing of different pests have

been reviewed from time to time and are mentioned here (Gaur and Khetrapal 1994; Khetrapal and Kumar 1995; Agarwal and Sinclair 1997; Khetrapal 2004).

27.2.1 Conventional Methods

Some classical diagnostic methods have been standardized in the last century and have been applied to seed samples for a long time to test seeds.

27.2.1.1 Visual Inspection of Seeds

The dry seeds are examined either by naked eye or with the help of hand lens, not only for the presence of plant debris, sclerotia, nematode galls and smut balls, etc., which are classified as 'inert matter' in purity analysis of seed testing, but also for symptoms which the pathogen might have produced on the surface of the seeds. The various types of symptoms are as follows:

Seed Rot and Necrosis

A number of fungi associated with seeds cause seed rot during germination. *Fusarium avenaceum* is a common seed rot-causing fungus in a variety of hosts. Other species like *F. culmorum*, *F. moniliforme* and *F. semitectum* are also known to cause seed rot. *F. graminearum* causes seed rot in maize. The seed rot is also caused by *Alternaria porri* f. sp. *dauci* and *A. radiciana* in carrot, *Phoma lingam* in cabbage and *Mycosphaerella pinodes* in pea. Most of the seed-borne fungi do not penetrate deeper into the fleshy cotyledons and produce necrotic lesions on the surface of the seeds.

Shrunken Seeds

Many pathogens which are not seed-transmitted yet attack the foliage often impair photosynthesis resulting in poor development of the seeds, viz. *Puccinia graminis*, *Albugo candida*, *Sclerospora philippinensis*, *S. sorghi* and *Peronospora destructor*. Halfon-Meiri (1970) reported the reduction in seed size in crucifers and chickpea due to *Phoma lingam* and *Ascochyta rabiei*, respectively. The size of the seeds is drastically reduced due to *Septoria linicola* in flax and *Drechslera teres* in barley. Wheat seeds infected with *Alternaria triticina* get shrivelled, whereas those seeds infected with *Drechslera sorokiniana* get reduced in size and weight.

Fructifications

The seeds of coriander become hypertrophied due to infection of *Protomyces macrosporus*. Species of *Phoma* and *Macrophomina* produce fructifications on the surface of seeds. Johnson and Lefebvre (1942) for the first time observed the crusts of oospores of *Peronospora manshurica* on the surface of soybean seeds and concluded that downy mildew in soybean is seed-borne in nature. The seed transmission of the pathogen was established by Jones and Torrie (1946).

Sclerotization

The ovary gets converted into a hard structure known as sclerotia. Species of *Claviceps* often produce sclerotia in the majority of cereals and other grasses. The shape and size of the sclerotia depend largely on the host. The sclerotia of *Claviceps purpurea* produced on *Secale cereale* are stout and up to 2–3 cm long, while the sclerotia of the same pathogen on *Phleum pratense*, a grass, are very tiny. *Gloeotinia temulenta* is a closely related genus and causes blind seed disease in *Lolium perenne*.

Seed Discolouration

Fungal infection on seed coat in many crops causes discolouration which results in not only poor seed quality but also reduced market value.

Superficial Necrotic Lesion

The pathogen causes black brown or grey necrotic lesions. Ram Nath et al. (1970) described these symptoms on the seeds of *Phaseolus aureus* infected with *Fusarium equiseti*, *F. semitectum* and *Macrophomina phaseolina*. The white streaks radiating from the embryo part of maize seed are often associated with *F. moniliforme*, while the black streaks indicate the presence of *Drechslera maydis*.

Fungus Coating

Kondo and Okamura (1927) reported the fungus coating of *Drechslera sorokiniana*, *D. teres* and *D. oryzae* on the seeds of cereals, barley and rice, respectively. Heavy sporulation of *Fusarium graminearum* causing head blight in wheat also makes the kernels pink or orange in colour.

Pigmentation

Some of the pathogens or even the saprophytes produce pigmentation on the surface of the seeds, which indicates their presence. However, the ability of the fungus to produce pigmentation varies from strain to strain. It is also not necessary that all the infected seeds show pigmentation. Ou (1963) reported that rice seeds infected with *Tricochoniella padwickii* give a pink stain. Brinkman (1931) observed a red discolouration around the micropyle of bean seeds infected with *Stemphylium botryosum.* This has been termed as ‘red nose’. Kilpatrick (1957), Johnson and Jones (1962) and Laviolette and Athow (1972) observed purple stain on the surface of soybean and cluster bean seeds due to *Cercospora kikuchii*. Similarly, *C. sojina* results in grey or brown discolouration of soybean seed coat. Likewise, *Macrophomina phaseolina* (black spots) and *Colletotrichum truncatum* (greyish discolouration with minute black specks) also occur on soybean seeds. The brown spots on the seeds of pea and beans often indicate the presence of *Ascochyta pisi* and *Colletotrichum lindemuthianum*. Dastur (1932) reported the blackening of the germinal end of wheat seeds due to *Alternaria tenuis*. The disease is commonly known as black point. *Drechslera sorokiniana* and *Curvularia lunata* are also associated with black point of wheat.

27.2.1.2 Washing Test

Two grams of seed is taken in a test tube with 10 ml of water and shaken for 10 min on a mechanical shaker. The suspension is examined as such or the suspended spores are concentrated by centrifuging at 3000 rpm for 15–20 min. The supernatant is discarded, and the spores are again suspended in 2 ml of lactophenol (a mixture of lactic acid, phenol, water and glycerol in the ratio of 1:1:1:2). This suspension is then examined under the microscope for the presence of spores, conidia and other fructifications. Rice (1939) suggested the use of a haemocytometer for the semi-quantitative estimation, where the spore load per gram of seed can be calculated with the help of the following formula:

$$\frac{N \times V}{0.0001} = N \times V \times 10{,}000$$

$$\left(0.0001 \text{being the value of fluid in central square of haemocytometer}\right)$$

Where,

N is the number of spores in the central square
V is the value of mounting fluid added to the sediment
W is the weight of seeds

So, the spore load/g of seed will be:

$$\frac{N \times V \times 10{,}000}{W}$$

Merits: The method is good for quick detection of externally seed-borne mycoflora.

Oospores and chlamydospores can also be detected by this method.

Demerits: Internally, seed-borne pathogens cannot be detected by this method.

27.2.1.3 Seed Soak Method

The seeds are soaked in a solution of sodium hydroxide or potassium hydroxide to soften the tissues. The method is widely used for the detection of pathogens of loose smut, Karnal bunt and paddy bunt diseases.

Detection of Loose Smut of Wheat

The disease is caused by *Ustilago segetum* var. *tritici*. In infected seeds, the mycelium of the pathogen is present in the embryo.

About 1500 wheat seeds are soaked for 20 h at 25 °C in 5 per cent solution of NaOH containing 0.15 g of trypan blue per litre. The soaked seeds are then transferred to a set of two sieves arranged in an ascending order of 10 and 50 mesh size so that the 10 mesh size sieve is at the top. A stream of hot water at 60 °C is passed over the soaked seeds to separate the embryos from the chaff. Most of the embryos are collected in the lower sieve, while the chaff remains on the top sieve. The embryos thus separated are once again washed with water to remove the traces of

NaOH and later transferred to ethyl alcohol for 2 min for dehydration. The dehydrated embryos are transferred to a funnel, the stem of which is connected to a rubber tube with a stopcock. A 3:1 mixture of lactophenol and water is added. The embryos float and the chaff sinks which can be drained easily into a beaker. The process is repeated three to four times till the embryos are free from chaff. The clean embryos are then gently heated avoiding vigorous boiling in a fresh solution of lactophenol to remove the excess of stain, if any. Once the lactophenol cools down, the embryos are ready for examination. The embryos are examined at 12x or 25x magnification of a stereo-binocular microscope. The infected embryos have bluish thread-like mycelium (with uniform thickness and swellings) which may be present in scutellum, plumule bud or the whole embryo.

The method was initiated by Skvortzov (1937) who dissected out the embryos and then macerated them with NaOH and stained with aniline blue. But, significant modifications were made by Simmonds (1946), Russel (1950) and Popp (1958). However, Khandzada et al. (1980) after evaluation found that the Scottish method is simple, convenient, economical and accurate. It gives accurate and precise information for predicting the incidence of the disease.

Merits: The method can be used for the detection of downy mildew mycelium as well.

Demerits: The method is cumbersome and time-consuming and requires a trained eye to detect the presence of mycelium in the embryo.

Detection of Karnal Bunt (*Neovossia indica*) in Wheat and Bunt (*N. horrida*) in Paddy Seed Lot

Wheat seeds are soaked in 0.2 per cent solution of NaOH for 24 h at 25 °C for the detection of Karnal bunt. The solution is decanted, and the swollen seeds are spread over a blotter to remove excess of moisture. These seeds are visually examined for brown, dull or shiny black discolouration. Seeds with such discolouration are further examined under a stereo-binocular microscope. Seeds with shiny jet-black discolouration are bunt infected, while others are not. The infection can be confirmed by rupturing the seed with the help of a needle in a drop of water. A stream of bunt spores is released from the shiny jet-black discolouration. This method can also be used to differentiate between Karnal bunt and black point of wheat, as it is difficult to differentiate between the two diseases particularly when the Karnal bunt infection is in the incipient stage. Agarwal and Srivastava (1985) suggested that this method can also be used for the detection of paddy bunt.

Merits: This method is good in clearing the seed surface and increases the colour intensity between the infected and healthy seeds.

Demerits: The number of infected seeds is an indication of the remnants and not of the huge quantity of teliospores and fragmented seeds lost in the process of soaking, decantation and drying.

27.2.1.4 Incubation Methods

The seeds are sown in Petri plates either on moist blotter or on any other suitable synthetic or semi-synthetic medium and then incubated for 5–7 days at recommended conditions of temperature and light.

Blotter Method

The method was developed by Doyer (1938) which was later included in the International Seed Testing Association Rules of 1966. Usually, 400 seeds per sample are placed on three layers of blotters moistened with tap water in plastic Petri plates. If the seeds are small in size, 25 seeds per plate and if they are large in size then 10 seeds per plate equidistantly are to be plated. The plates are incubated at 20 °C for 7 days under diurnal cycles of 12-h light and 12-h darkness. Normally, the NUV light should be used, but in most of the Southeast Asian countries, black light or NUV tubes are not available, and hence, normal day light tubes can be used with the same efficiency. The distance between the tubes and the plates should be 40 cm. After incubation, the plates are examined under a stereo-binocular microscope for the presence of fungi like *Alternaria*, *Curvularia*, *Drechslera*, *Fusarium*, *Phoma*, etc.

Merits: Blotter test is a combination of in vitro and in vivo principles of investigation. The stereo-binocular microscope enables the observation of fungi developed on their host, in situ, undisturbed and in a condition of natural growth.

Demerits: Obligate parasites cannot be observed by this method. Fast-growing saprophytes often cover the whole seed and create difficulty in proper study of the pathogenic fungi.

2,4-D Method

This is a modification of the blotter method. During incubation, seeds germinate and obstruct observation. To overcome this, 2,4-D is used to make the identification of seed mycoflora easy as the chemical inhibits seed germination. Instead of water, the blotter is soaked in 0.2 per cent solution of 2,4-dichlorophenoxy acetic acid. Incubation and other conditions remain the same as in the blotter method.

Deep-Freeze Method

This method was developed by Limonard (1966). After plating the seeds as in the blotter method, the Petri plates are incubated first at 20 °C for 48 h to induce germination and then transferred to –20 °C in a deep freezer for 24 h and again brought back to 20 °C under NUV or daylight tubes for another 3–5 days. Fungi like *Fusarium* and *Septoria* in cereals, *Phoma* in sugar beet and *Pyrenophora graminea* and *P. teres* in barley can be detected by this method.

Merits: The dead seeds act as a natural substrate for the growth of fungi.

Demerits: The method involves the problem of shifting the plates from 20 °C to –20 °C and then back to 20 °C. An additional equipment (deep freezer) is required.

Agar Plate Method

Preparation of Media

Generally, potato dextrose agar (PDA) is used as a substrate. About 200 g of peeled and sliced potato is boiled for 15 min in 500 ml of water. The contents are then filtered through a muslin cloth. The potato slices are thrown off, and the starch extract is kept separately. Now 20 g of agar is boiled in another 500 ml of water. Then the starch extract and the agar solution are mixed in a 1 L flask/beaker. Finally, 20 g of dextrose is added, and the volume is made up to 1000 mL. Recently, some companies are marketing prepared PDA in powder form. Only 39 g of this powder is dissolved in 1000 ml of water to get 1 L of PDA. This PDA is divided into four 250 ml flasks and plugged with non-absorbent cotton plug and then autoclaved at 20 lbs. psi for 20 min.

The glass Petri plates should be washed properly, followed by sterilization which either can be done in a hot air oven at 160 °C for 8 h or may be wrapped in a butter paper and then autoclaved at 20 lbs. pressure psi for 20–25 min.

Preparation of Plates

Keep the laminar air flow on for 10 min before pouring the media into the plates. When the temperature of the PDA comes down to around 50 °C, carefully lift the lid of the previously sterilized Petri plate and pour about 20 ml of the PDA into it. Stack the plates one over the other and allow the PDA to solidify. These plates should be used after 24 h. Plates showing any contamination should be discarded.

Sowing the Seeds

Pretreatment of seed is essential in the agar plate method. The seeds should be pretreated with 0.1% $HgCl_2$ solution followed by washing with distilled water twice to remove the traces of mercury. The seeds are picked up with sterilized forceps and placed on the PDA.

Normally, ten seeds are placed equidistantly, but if the seeds are of bigger size, five seeds per plate may be placed. If the studies are to be conducted for some storage fungi like *Aspergillus* and *Penicillium*, 180 g of sodium chloride per litre of PDA is to be added, since a high-pH, low-water activity (low-aw) medium provides a more representative growth medium. The plates are incubated at 20 °C for 5 days under alternate cycle of light and darkness. This method was used for routine seed health testing of flax in Ulster without pretreatment of seeds, and so it is popularly known as the Ulster method (Muskett and Malone 1941). Fungi such as *Ascochyta* spp., *Trichoconiella padwickii* and *Septoria nodorum* can be easily detected by this method. In the agar method, the identification is based on colony characters only which may be sometimes misleading.

Merits: A large number of plates can quickly be examined as the identification is based on colony characters.

Demerits: It is a complicated, cumbersome and expensive method as compared to the standard blotter method. Only an expert analyst acquainted with colony characters can identify the pathogens.

Guaiacol Method

A quantity of 0.124 g of guaiacol and 0.146 of coumarine is dissolved in 20 ml of distilled water which is then added to 980 ml of distilled water. Having added 5 g of agar to this, the medium is autoclaved as described in the agar plate method. As soon as the medium cools down to about 50 °C, 0.5 g of streptomycin is added to it, which is now poured into the sterilized glass Petri plates aseptically. The plates are stacked one over the other, and the medium is allowed to solidify. Paddy seeds are pretreated with 0.1% $HgCl_2$ solution and then washed twice with distilled water to remove traces of mercury and then sown on the medium and incubated for 4 days. A pink- to red-coloured halo develops around the infected seeds which can be seen by the naked eye. The numbers of infected seeds are counted, and the result is reported in percentage. The method was developed by Kulik (1973).

Merits: Since the method is macroscopic, a large number of samples can be tested in a short period.

Demerits: The method is cumbersome, expensive and specific to *Pyricularia oryzae*.

Hold-Fast Method

Prepare 1.2% water agar medium, and after sterilization, pour it into the plates as described in the agar plate method. Place the untreated or chlorine-treated sugar beet seeds on the medium and incubate at 20 °C for 5–7 days. After incubation, hold-fast-like structures (swollen hyphal tips) develop which can be seen from the backside of the plate under the microscope. Development of hold-fast indicates the presence of pathogen. The method was developed by Mangan (1971). The results are reported in percentage.

Merits: The technique is comparatively good than the blotter method as the agar medium shows characteristic structure.

Demerits: Slow-growing fungi may not appear appreciably and, therefore, may be suppressed by fast-growing fungi. The test is also expensive on account of agar and glass plates. The method is specific for *Phoma betae*.

The conventional methods are mostly based on interpretation of visual symptoms, culturing and laboratory identification. These methods require sound taxonomic expertise and are very time-consuming. In many cases, they are not reliable, and it is almost difficult to interpret results with hidden symptoms.

27.2.2 Molecular Methods

Molecular methods are able to provide precise, reliable and reproducible results rapidly. They facilitate early disease management decisions. Biochemical, immunological and nucleic acid-based methods have distinct advantages. These are useful in the identification of obligate parasites. The availability of antisera, primers and

commercial kits is useful for on-site detection during field surveys for assessing the distribution of existing pathogen as well as new/introduced pathogens through seeds/material. Among the tools available for pathogen detection, nucleic acid-based techniques are the most accurate and least time-consuming. The initial establishment of a molecular seed pathological laboratory may be expensive, but when samples are examined on a large scale using molecular methods, the cost becomes quite low.

27.2.2.1 Detection of Seed-Borne Bacteria and Viruses

Serological techniques are more applicable than other methods to detect bacteria in seeds because of its specificity, rapidity, quick detection and identification ease of testing a large number of samples. Some of the serological tests used for detecting and identifying seed-borne bacteria include agglutination test, immunofluorescence test, immunodiffusion, direct double diffusion, Ouchterlony double diffusion test and enzyme-linked immunosorbent assay (ELISA). Presently, ELISA is the most widely used method for the serological detection of bacteria as these are more sensitive, use less antibody and can be employed for simultaneous handling of a large number of samples in routine testing. The most widely used ELISA is double-antibody sandwich (DAS), while another one is known as direct antigen-coated (DAC) ELISA (Marcinkowska 2002).

Electron Microscopy for Detection of Viruses

Electron microscope is an optical instrument in which a beam of electrons is used to form a greatly enlarged image of an extremely small object through a series of electromagnetic lenses. Transmission electron microscopy (TEM) can be used directly to detect the presence of virus in the plant tissue. It reveals the shape and size of the virus particle. The shape and size of a virus particle give an idea of the group to which it may belong.

DAS ELISA for Detection of Viruses

The ELISA technique invented by Clark and Adams (1977) has become the most widely used serological method for routine detection of plant viruses. The enzyme most commonly used in plant virus detection is alkaline phosphatase which dephosphorylates the colourless substrate para-nitro phenyl phosphate producing the bright yellow para-nitrophenol (Dadlani et al. 2013) (Tables 27.1 and 27.2).

27.3 Seed Certification

Seed certification for a crop comprises legal norms to be qualified for ensuring genetic identity, physical purity, germinability and freedom from seed-transmitted pathogens and weeds (Khetrapal and Chalam 2012). The International Seed Testing Association (ISTA) and Association of Official Seed Certifying Agencies (AOSCA) have introduced minimum seed certification standards. The certification procedures make sure that the genetically pure and quality seed are produced in the field. Care is also taken during harvesting, processing, storage and finally inspection in the market.

Table 27.1 External symptoms of seeds associated with the infection of some seed-borne fungal pathogens

Host	Symptoms	Pathogen
1. *Triticum vulgare*	Fungus coating	*Fusarium graminearum*
	Shrivelled seeds	*Septoria nodorum*
	Shrunken seeds	*Puccinia graminis*
2. *Oryza sativa*	Fungus coating	*Drechslera oryzae*
	Pink stain	*Tricochoniella padwickii*
3. *Hordeum vulgare*	Fungus coating and reduction in seed size	*D. teres*
4. *Zea mays*	White streak radiating from embryo	*Fusarium moniliforme*
	Black streak radiating from embryo	*Drechslera maydis*
	Shrunken seeds	[a]*Sclerospora philippinensis*
	Seed rot	*Fusarium graminearum*
5. *Sorghum vulgare*	Shrunken seeds	[a]*Sclerospora sorghi*
6. *Glycine max*	Purple stain	*Cercospora kikuchii*
	Crusts of oospores	*Peronospora manshurica*
7. *Phaseolus aureus*	Black, brown or grey	*Fusarium equiseti*
	Necrotic lesions	*F. semitectum*
8. *Dolichos lablab*	Red discolouration around micropyle (red nose)	*Stemphylium botryosum*
	Brown spot	*Colletotrichum lindemuthianum*
9. *Pisum sativum*	Brown spot	*Ascochyta pisi*
	Seed rot	*Mycosphaerella pinodes*
10. *Cicer arietinum*	Reduction in seed size	*Ascochyta rabiei*
	Shrivelled seeds	*Botrytis cinerea*
11. Crucifers	Reduction in seed size and seed rot	*Phoma lingam*
12. *Daucus carota*	Seed rot	*Alternaria radicina*
13. *Allium cepa*	Seed rot	*Alternaria porri*
	Shrunken seeds	[a]*Peronospora destructor*
14. *Linum usitatissimum*	Reduction in seed size	*Septoria linicola*
15. *Coriandrum sativum*	Hypertrophy	*Protomyces macrosporus*

[a]Not seed-borne

Certification of seed-borne pathogens is followed only as and when required depending on the impact of the pathogen on yields. The objective of developing certification programmes for seed-transmitted pathogens is to ensure that the level of infection of the seed lot which is to be given to the growers is acceptable and can be used for producing seeds of a variety as it is free from pathogens. Apart from it, the seed material under international exchange needs to be certified as pest-free, to minimize the risks associated with the introduction of exotic pests or virulent strains/biotypes to an area in which they have not been previously reported (Maury et al. 1998).

A tolerance limit for the diseases has to be fixed for the quality control of seeds. The tolerance limit refers to the inoculum threshold that can be tolerated, and to

Table 27.2 Morphological seed abnormalities correlated with infection of some seed-transmitted viruses

Plant species	Seed abnormality	Probable responsible virus
Allium cepa	Reduced size	*Onion yellow dwarf virus*
Arachis hypogaea	Small size, discoloured seed coat	*Peanut mottle virus*
Cucumis melo	Poorly filled, deformed	*Squash mosaic virus*
Cucurbita pepo	Poorly filled, deformed	*Squash mosaic virus*
		Soybean mosaic virus
Glycine max	Discoloured seed coat	*Soybean stunt virus*
Hordeum vulgare	Small size, shrivelled, light weight	*Barley stripe mosaic virus*
Lactuca sativa	Light weight	*Lactuca mosaic virus*
Lupinus luteus	Large or sharp edged	*Bean yellow mosaic virus* (Lupid narrow leaf strain)
	Large size	*Cucumber mosaic virus*
Lycopersicon esculentum	Necrotic, blackened	*Tobacco mosaic virus*
Vigna radiata	Wrinkled, shrivelled	*Mungbean mosaic virus*
Pisum sativum	Wrinkled, greenish grey seed coat	*Pea early browning virus*
	Cracked but 'healed' seed coat, small size or abnormal shape	*Pea seed-borne mosaic virus*
Vicia faba	Peripheral brown necrosis of seed coat	*Broad bean stain virus*
	Discoloured seed coat	*Bean yellow mosaic virus*
Vigna unguiculata	Shrunken, shrivelled	*Cowpea aphid-borne mosaic virus*

determine it, one needs to collect detailed epidemiological information on the seed transmission rate, the degree of susceptibility of various cultivars, the rate and intensity of field spread in relation to climatic conditions, the resulting percentage of infected plants in the field and yield reduction (Maury et al. 1985). However, for most of the seed-borne pests/diseases, not much serious work has been done in this direction (Dadlani et al. 2013).

27.4 Conclusion

Unhealthy seeds have either external symptoms or morphological seed abnormalities. Mainly different types of conventional methods followed by molecular methods are employed for seed health testing. The seed soak conventional method is widely used for the detection of fungal pathogens of loose smut, Karnal bunt and paddy bunt diseases. The conventional methods require sound taxonomic expertise and are very time-consuming. In many cases, they are not reliable, and it is almost difficult to interpret results with hidden symptoms. Presently, ELISA is the most widely used method for the serological detection of bacteria and viruses as these are more sensitive, use less antibody and can be employed for simultaneous handling of a large number of samples in routine testing. These all above-mentioned techniques facilitate early disease management decision, but nucleic acid-based techniques are the most sensitive tool for seed health testing.

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Non-parasitic Seed Disorders of Major Agricultural Crops

28

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Abstract

Non-parasitic seed disorders are the abnormalities in seeds caused by environmental and nutritional stresses, alterations in genetic makeup, or some mechanical injuries caused to the seeds during harvesting and handling of the crop. These disorders are not caused by any infectious agent, but they can make the seed susceptible to infection by many pathogens. This chapter describes the identification, cause, and management of seed disorders of field and vegetable crops. Disorders caused by environmental stresses like high or low temperature, humidity, moisture, frost, and drought are generally irreversible; however, their incidence can be minimized by opting suitable sowing and harvesting time along with providing adequate seed storage conditions. Disorders like yellow berry, hollow heart, and marsh spot, which are caused by the deficiency of different nutrients, can be avoided by providing a balanced nutrition to the seed crops by soil and foliar application of the deficient nutrients. Mechanical injuries during threshing and seed processing also result in many seed disorders. Baldhead or snakehead in beans, for example, is a common disorder that occurs due to injury at any stage including harvesting, threshing, cleaning, or during seed sowing operations. Disorders caused by genetic mutations can be reduced by breeding for stress-resistant cultivars of various crops by gene transfer and devising efficient screening protocols to screen genotypes against various abiotic stresses.

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28.1 Introduction

Non-parasitic seed disorders, as the term indicates, are not caused by any infectious agent and hence cannot be transmitted from one plant to another. However, they can often make the seed susceptible to infection by pathogens. They are caused by non-living abiotic factors such as lack or excess of something that supports life or by the presence of something that interferes with life and cause a deviation from normal growth. They may cause morphological or physiological changes in plant from the normal pattern and are caused by an external factor. Most of such disorders are irreversible once they have occurred and can affect plants in all the stages of their growth. Plants' response to the cause of disorder varies widely from little reaction to death. There is generally a clear line of demarcation from damaged and undamaged tissue. Stress is the major factor responsible for the appearance of various seed disorders. Stress may be induced by environmental or nutritional factors. Apart from stress, genetic alterations in plants and seed injuries caused by various factors also cause seed disorders.

28.2 Disorders Caused by Environmental Stress

Environmental stress is the major cause of many such disorders. It acts as a complex of various interacting factors that contribute in varying degrees to the overall stress. Seed crop encounters several abiotic stresses such as temperature, moisture, cold, frost, sunscald, air pollution, soil pH, salinity, sodicity, drought, water logging, and nutritional imbalances at different stages of crop growth in the field, such as vegetative state; flowering; seed setting; seed maturity; and postharvesting operations including harvesting, threshing, and processing. It reduces the vegetative growth of plants, thus reducing the photosynthetic activity of plant, which has a primary effect on seed number. Water stress at pollination and early seed development affects pollen transfer and fertilization in corn and abortion of developed kernels in the early stage. Decreased photosynthesis in maize plants results in the inability of stressed ovaries to utilize sucrose efficiently, leading to embryo abortion (Aslam et al. 2015). Water or nutrient stress at seed development also reduces seed quality. Catfacing is an abiotic disorder in tomato and other solanaceous crops, which occurs when temperatures drop below 0 °C at the time of flowering and fruit setting. This disorder may also be due to excess heat or 2,4-D injury or erratic soil moisture. Excess of nitrogen has been found to aggravate this disorder. The symptoms appear as deep indentations in the blossom end of the fruit. In such fruits, the invasion of fungus may take place, resulting in seed infection. Puffiness occurs due to cool temperatures or high nitrogen and low potassium in tomato, which results in incomplete pollination, fertilization, or poor seed development in some susceptible tomato cultivars.

There is a wide variation in the effects of stress on seed development and seed quality, leading to severe reduction in vigour and viability. Seeds developing inside a wet fruit at the seed maturity stage can lead to precocious germination due to high humidity, which is a character of poor seed quality. In many cases, the seeds that are

produced under stress conditions are not impaired in their germination capacity, although seed size may be reduced. Lettuce seeds that mature at higher temperatures subsequently set higher temperature limits for germination than those that mature at lower temperatures.

The genetic characters of a host species or a host variety are also influenced by environmental conditions such as day length, light quality, season of maturation, altitude, and other factors during seed germination, development, or maturation, and that is why the environmental requirements for seed production of a particular crop or variety must be taken into consideration in order to obtain high-quality seed and minimize the problems of dormancy or other ill effects of the environment. The major environmental stresses that influence the seed development and quality are temperature, humidity, water logging, frost, fog, drought, etc.

28.2.1 High-Temperature Effects

Generally, during storage, self-heating of seeds takes place. The air-dried seed may tolerate rather higher temperature of self-heating, whereas the moist or insufficiently mature seed may lead to serious damages of seeds due to self-heating during storage. Such seeds may become brownish, wrinkled, and the embryo of such seed is weakened. Seedlings developed from such seeds are abnormal and may not develop root hairs, or the embryo may be killed.

Pulses are particularly sensitive to heat at the full bloom stage in temperate regions. A few days of exposure to high temperatures (30–35 °C) causes heavy yield loss due to flower drop and pod abortion. There is marked reduction in yield after exposure to high temperature during seed filling. It can reduce seed set and seed weight and accelerate senescence. High temperature due to excessive heat during the drying of pea seed may lead to loss of viability, internal cracks, split seed coats, and discolouration.

An increase in temperature has a pronounced effect on both developing and mature soybean seeds. Expression of genes that regulate seed development and carbohydrate metabolism is severely reduced at high temperature, thus decreasing the quality and yield of many seeds. The total oil (particularly linolenic acid), carbohydrate, protein, and phosphorus contents decrease, while the oleic acid content increases at high temperatures.

Heat canker is the direct result of the high temperatures of the dry soil surface in immediate contact with the tender young stems in carrot. In most cankered plants, the stem breaks sooner or later and the plant dies. The amount of injury is influenced by the temperature of soil surface and the age of the plant.

Sunscald is another physiological disorder encountered in onion crops due to rise in the temperature. The disorder is characterized by greyish, watery tissues of one or more layers or scales, or strangling of the neck, which makes them appear translucent. Sometimes the condition affects all the layers, but most often, only the second and third fleshy scales are affected. During hot and sunny weather, sunscald may be noticed on green or maturing tomato/chilli fruits. This disorder appears as

white or light tan discolouration of the fruit that has been exposed to sun and affects the seed quality adversely. The disorder is also visible on bean pods as rusty appearance on the pod surface.

Scorching can be a major physiological disorder especially in hot climates.

Heat stress occurs when the plant cannot take up water fast enough for its needs. Pulses are particularly sensitive to heat at the full bloom stage. A few days of exposure to high temperatures (30–35 °C) causes heavy yield losses through flower drop and pod abortion, causing marked reductions in seed yields.

28.2.2 Low-Temperature Effect

Low-temperature adversely affects the crop. The losses are significant when such condition prevails during blossoming or seed setting and at the maturity of the crop. Most chickpea cultivars, in Australia, continue to flower but fail to set pods when the temperature falls below 10 °C at flowering. It is due to the reduced ovule fertilization, associated with decline in pollen tube growth and ovule viability at a low temperature, leading to poor seed set in chickpea. This phenomenon is also found in soybean cultivated at high altitudes. However, this phenomenon varies with cultivars (Thuzar et al. 2010; Patra et al. 2011).

28.2.2.1 Chilling Effect

Exposure of plants for longer duration at a low temperature will result in chilling stress and can retard growth of the plant. Cold conditions cause abortion of flowers and immature pods and reduce seed yield in brassica seed crops. In sunflower, seed lots containing a high percentage of hulled seed or immature seed, such as resulting from an early frost, tend to deteriorate in storage, affecting oil quality. Under Australian winters, reproductive organs, mainly flower buds and flowers in pulses, are most susceptible to cold or chilling injury, resulting in reduced seed yield and poor seed setting. The germination stage of soybean consists first of a very fast uptake of water (imbibitional phase), followed by a much slower uptake of water (osmotic phase). Chilling during the first phase can cause severe problems because the imbibed water is needed to rehydrate the cotyledons and embryo to the point that cell membranes become functional. Cold temperatures interfere with proper hydration of those membranes. The imbibitional phase typically is not very long (less than 24 h) and can occur with relatively little soil moisture since the seed is dry. Thus, getting a cold rain 24 h after planting can lead to chilling injury in soybean and lowers crop stand. A study has shown that when the soybean seed coat was removed, imbibition injury occurred within 30 min. With a seed coat, imbibition is slower and a longer exposure would be needed for chilling injury to occur (Bramlage et al. 1978).

28.2.2.2 Frost Effect

Frost injury in wheat during the milk to the soft dough stage is characterized as fairly plump kernels covered with small blisters confined to the back of the kernel or sometimes wrinkled kernels (flaked) in which wrinkling has been caused by frost, while

the kernels are still immature and are damaged due to frost. In case of wrinkled or flaked kernels, it is confusing if the damage is due to frost or the bran coat has been rubbed off during handling. Flowering, early stage of pod formation, and seed filling stages are the most sensitive stages to frost injury in winter pulses. Among the pulses, field pea is most susceptible to frost injury during the reproductive stage. The siliquas of rape, *Brassica napus,* may, after frost, still look normal, but the seeds inside are brown, shrunken, and dried up. Yield losses may go up to 100%. Frost may cause reduced germination in seeds of lupin and beets. Early autumn frost injuries destroy the maturing seed of crucifers, particularly in cauliflower. In Norway, frost damage is common in seeds of barley, oats, and rye. Seedlings from damaged seeds often have a stunted, swollen root, and a curled sprout.

28.2.3 Humidity Effect

The normal water content of seeds varies from one plant species to another and depends greatly on the chemical composition of the seed. The maximum moisture limit for seeds of grass, beet, and spinach is 15%, but for cabbage and radish, it is 9%. Onion and flax seed must be dried down to 8% water content to keep well in storage. Oil seed sunflower should not be stored above 10% moisture during the winter and 8% during the summer. Non-oilseed sunflower should not be stored above 11% moisture during the winter and 10% during the summer. Sunflower can be stored for short periods at 12% with an adequate airflow to keep the seeds cool.

28.2.3.1 High-Humidity Effect

High humidity during a wet harvest causes discoloured seed coating, giving the seed a weathered appearance and a reduced test weight in flax seeds (Gaylon et al. 2017). In Denmark, grey discolouration of radish (*Raphanus sativus*) and white mustard (*Sinapis alba*) seed is very common. In the field, this disorder occurs only when the crop is close to maturity and the weather is humid. The breaking of epidermis may facilitate invasion of *Alternaria* species, which may penetrate deep into the seed. *Alternaria brassicae* is particularly frequent in white mustard, while *Alternaria raphani* is frequent in radish seeds. In cucurbitaceous crops like muskmelon, a high humidity condition remains within the fruit. If such fruits are left after ripening for an excessive period in the field, they begin to decay and the seeds within the fruits loose seed viability (Welbaum and Bradford 1988; Welbaum et al. 1990). It also causes the appearance of swollen seeds with split seed coats or osmotically distended seeds, a condition known as “fishmouth” in cucurbits (Welbaum 1992, 1999).

28.2.3.2 Low-Humidity Effect

In corn, moisture stress or low humidity causes nutritional deficiencies, which usually delay silking more than tassel emergence and pollen shedding. Stressed plants at high temperatures during seed filling can diminish seed set, seed weight, and accelerate senescence and reduce yield. Boyle et al. (1991) reported that the embryo abortion in corn is due to the reduction in the supply of sucrose resulting from decreased

photosynthesis in stressed plants. Hollow heart disorder in pea seeds is due to the low humidity effect. Seeds with this disorder are usually normal in-outward appearance but have sunken or cracked areas in the centre of the cotyledon face. The cells lining the hollow part are abnormal, and these are readily colonized by pathogenic and saprophytic fungi leading to the rotting of the cotyledons. This disorder occurs as a result of quick drying of immature seeds due to the high temperature during maturation of the seeds on the plant or by its drying in the immature stage (Perry and Howell 2007).

28.2.4 Drought Stress and Water Stress

It is a major constraint in all pulse-growing regions, particularly, in the low-rainfall areas. There are two major effects of drought on pulse productivity. The first is the failure to establish the desired plants stand and the reduction in growth and yield due to sub-optimal soil moisture availability. The cotyledons of pea and bean seedlings, raised under low-humidity conditions, may have transverse cracks and poor seedling emergence, causing the primary leaves to be small and deformed. The defect resembles mechanical damage. The second drought is the physiological drought, which results in necrotic spots in cotyledons and, in extreme cases, the killing of the entire embryo of seed as in lettuce due to moisture stress at a temperature of 32 °C and above over an extended period.

Flowering is the most sensitive stage to drought. Drought stress causes significant yield losses in chickpea and field pea. It is probable that the high sensitivity to drought during the reproductive stage is due to the lack of new root growth in pulse crops at this stage. Yield losses in chickpea due to terminal drought can vary between 30% and 60% depending on the location and climatic conditions during the crop season. In faba bean, potential yield loss may be up to 70% due to the drought effect (Lopez et al. 1996). In soybean, the drought effect may be experienced as crinkling of the seed coat due to desiccation during the seed maturation (Frederick et al. 2001).

Erratic moisture conditions can cause radial and concentric cracking on fruits of vegetable seed crops. This is a serious physiological disorder in tomatoes that makes them unmarketable and causes abnormal seed development inside the fruit. Disorders like cat facing, blossom scar, and puffiness in tomato and other solanaceous crops can be related to soil moisture variability (Vishunavat 2016). The cracks on fruits deteriorate the seed quality and make them accessible for invasion by the pathogens.

28.3 Disorders Caused by Nutritional Stress

Crops require essential nutrients (carbon, hydrogen, and oxygen), macronutrients (nitrogen, phosphorus, potassium, sulphur, calcium, and magnesium), and micronutrients (boron, chlorine, copper, iron, manganese, molybdenum, and zinc), which are either derived from the soil or from the environment. A proper balance of all the essential nutrients is very important for optimum growth and better yield of the crop. These nutrients play a vital role for the production of agricultural crops.

Seed crops have similar nutrient requirements as those of the grain/commercial crops. Where possible, nutrients that stimulate reproductive development can be supplemented with normal fertility regimes. Boron, for example, increases the number of fruiting sites and seed per pod in legumes such as pea and beans. Molybdenum is also essential for optimum nitrogen fixation of legume seed crops. In vegetable seed crops, split application of fertilizers at planting and before flowering increases the formation of reproductive structures. Generally, lower nitrogen and phosphorus helps in seed production, as it reduces vegetative growth. Disorders caused by deficiency of different nutrients are explained below.

28.3.1 Nitrogen

Soils having low nitrogen and high potash and phosphorus have nitrogen deficiency symptoms in cereal seeds. The deficiency symptoms are called "*yellow berry*" of wheat and is recognized by its softer, light-coloured, and starchy endosperm, which lacks the vitreous texture of a normal grain. Seeds affected with yellow berry have higher moisture content and a high level of starch but are deficient in protein content, and the grains have lower specific gravity than normal seeds. This disorder can be corrected by nitrogen application to the crop.

28.3.2 Calcium

Calcium deficiency in groundnut results in lower yield, darkened plumules in the seed, empty pods (pops), and reduced percentage of sound mature kernel. Sometimes the plants deficient in calcium stay green and produce infertile flowers and pegs. Ca deficiency in groundnut can be subdued by the soluble source of calcium like gypsum, although certain groundnut cultivars do not always respond to such calcium supplements. In acidic soil, lime incorporation into the pod zone before planting can correct soil acidity and simultaneously supply adequate calcium for maximum yield of small-seeded cultivars in groundnut.

28.3.3 Boron

Boron deficiency results in *Hollow heart* in peanut or soybean (Perry and Harrison 1973). The inner surface of the cotyledons is depressed and darkened, and the kernels are graded as damaged. In pea, seeds appearing normal may suffer boron deficiency; seedlings develop pale, stunted shoots without the plumule bud (Perry and Howell 2007). Boron deficiency in black gram seeds does not show any visible symptoms, but the yield may fall up to 50%. Boron deficiency can be easily managed by applying boron, which is required in small quantities in the form of borax, a white crystalline salt.

Fig. 28.1 Marsh spot in pea

28.3.4 Manganese

Mn deficiency is common in legume crops. It is also known as "*Marsh spot*" in late maturing cultivars of pea, faba bean, common bean, and many other crops in England. It is common in cultivars that possess simple and large starch granules in the cotyledons. The affected cells secrete a pigment into enlarged air spaces due to the depletion of protoplasmic contents (Agarwal and Sinclair 1996). Symptoms appear as extended internal brown necrosis in the seed, the central part of the cotyledon being affected (Fig. 28.1). In severely affected cotyledons, cracks may appear. Some seeds may also show brown sunken spots in the seed coat, and this necrosis continues into the cotyledons beneath. The internal symptoms are detectable after the seeds are sown. Seedlings raised from such seeds are weak and later develop poorly. The quick remedial measure for this problem is the foliage spray with a solution of manganese sulphate. For a long-term solution, manganese salts should be added into the soil.

28.3.5 Potassium

Potassium deficiency in cucumber results in the production of tapered seeds (Agarwal and Sinclair 1996). Some workers have also reported that Marsh spot in pea is also caused by K deficiency along with Mn deficiency.

28.3.6 Zinc

Zinc deficiency leads to shedding of leaves and flower buds and failure of seed pods to develop. The application of zinc fertilizers in addition to NPK helps to overcome the deficiency symptoms. This tends to increase the weight of filled grains, reduces the percentage of empty grains, and increases the ratio of grain to straw.

28.3.7 Copper

Copper in plants serves as a micronutrient and has effective fungicidal properties. Copper deficiency affects the formation of grains, seeds, and fruit much more than it affects the vegetable growth. The main reason for the poor development of seeds and fruits is that a high percentage of the pollens in copper-deficient plants are not viable. Copper deficiency in wheat causes low grain formation. High application rates of phosphate fertilizer may increase in copper deficiency. For the management of leaf and stem diseases, foliar applications of copper-based chemicals such as copper-oxychloride, cuprous oxide, copper sulphate, and copper carbonates are often applied and are found more effective than soil application due to the effective reduction of fungal and bacterial diseases and improved nutritional status in crops.

28.4 Hereditary Disorders/Genetic Abnormalities

Although this is an internal factor and not an external cause, a plant can show strange symptoms when its own genetic system is altered. These disorders are not economically important but may be confused with infectious diseases or chemical injuries. Genetic mutations result in a variety of colours of cob and pericarp in ears of field corn. The causes are unknown but are believed to be related to stressful conditions following pollination. A report from Texas, for example, suggests that the silk-cut symptom occurs quite frequently in areas of south Texas prone to late-season drought stress (Odvody et al. 1997). Certain inbred lines in maize have a weak pericarp. This gives an appearance of "popped kernels" as irregular breaks at the crown of the kernels (Fig. 28.2). The other disorder is "silk cut," characterized by a horizontal cut or split in the pericarp over the sides of the kernels (White 1999). This breaks the seeds at the weakest points, exposing the starch of the endosperm,

Fig. 28.2 Popped kernel in maize

which is liable to be attacked by the fungi. This disorder is worst in yellow-seeded cultivars. The same type of disorder is also found in certain bean cultivars where the cotyledons are exposed due to split of seed coat.

28.5 Disorders Caused by Seed Injuries

Seeds suffer from various injuries such as rain injury, hail injury, chemical injury, mechanical injuries, and insect injuries. These injuries may occur in seed crop in the field or after harvesting or during processing operations. Many umbelliferous crops such as carrot, coriander, dill, and fennel often produce normal looking but embryoless seeds in which the embryo is replaced by a cavity, while the endosperm and seed coat remain undamaged. Seeds that have not matured properly or are physiologically weak and fail in proper development of a protective seed coat may give easier access to pests and pathogens, interior of the seed.

The mechanical damages during harvesting, postharvesting operations, (threshing, cleaning, and processing), transportation, and storage cause injuries in the seed. These injuries are generally minute and invisible to the unaided eyes or are easily visible as damages to the seed coat or internal tissues, adversely affecting the embryo of the seed leading to loss in seed germination, viability, loss of capacity in the regulation of water content of the seed, increase susceptibility to invasion by microorganisms, and to phototoxic effect. Injured seeds are often not capable of germinating into normal seedlings. During threshing, leguminous seeds are particularly sensitive to thresher injury, which, in beans, may produce up to 30% abnormal seedlings.

Baldhead or snakehead is caused by mechanical injuries in beans. This may occur at any stage of the seed threshing, cleaning, handling, or sowing operations. In baldhead, severely stunted and malformed bean seedlings are the typical symptoms. The epicotyls are fractured, just below the plumule. Once the injured seed is sown, even under ideal environmental conditions, it will result in abnormal plants. Generally, there is no or little plant growth above the cotyledons and no or tiny leaves are formed since the growing point has been killed. Similar damages occur in pea and clover. External damages are characterized by cracks of various sizes in the seed coat.

Flax seeds are also sensitive to threshing injury. The seed coat is brittle and may easily rupture when the seeds are knocked against the hard surface. Improper threshing practices lead to broken and bruised kernels in cereals, which may considerably reduce the market value of the grain. In soybean also, mechanical injuries during loading and transportation lead to substantial loss in germination.

28.6 Management of Abiotic Stresses

Abiotic stress can be reduced by choosing such a sowing time that the stress condition may be avoided at the sensitive crop stages in the season. One approach of dealing with stresses caused by extremes in the abiotic environment is to develop cultivars resistant to specific stress. Breeding and selection for resistance to these stresses is

often considered difficult due to the unpredictability of climatic conditions. The developmental stage of the plants and the duration of the stress have a strong influence on the effect of abiotic stress, together with the ability of the plants to tolerate the stress. Drought and heat escape through earliness in flowering, and maturity is the most widely used characteristic by breeders for crop improvement, especially in environments where drought affects the crop at its terminal growth stage.

Currently, lack of simple and accurate screening procedures to screen genotypes and breeding population for various abiotic stresses is the major bottleneck in the development of stress-tolerant cultivars. Germplasm for resistance to various abiotic stresses are not widely available to breeders. Future efforts are required to identify sources of desirable genes for transfer into adapted cultivars by conventional and/or biotechnological approaches to develop abiotic stress-resistant cultivars.

28.7 Conclusion

Non-parasitic seed disorders are usually caused by environmental and nutritional stresses, genetic mutations, mechanical injuries caused to the seeds during harvesting, threshing, and handling of the seed crops. These disorders are irreversible once they occur; however, they can be reduced by choosing appropriate sowing and harvesting time, providing balanced nutrition along with careful handling and storage of seed crops after harvest. Efforts should be made to identify sources of desirable genes for resistance to abiotic stresses in various crops and to develop efficient screening protocols for screening the germplasm against abiotic stresses.

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Storage Fungi and Mycotoxins

29

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Abstract

Seed is the basic unit of crop production and has greater contribution to environmental and cultural factors and is widely distributed in national and international trade. The seeds are found to be responsible for disease transmission because they carry a number of pathogens. The toxigenic fungal flora, existing in conjunction with food, largely includes genera *Aspergillus, Fusarium,* and *Penicillium* and, to a lesser extent, the genera *Alternaria, Claviceps,* and *Stachybotrys.* These economically important species of fungi produce significant mycotoxins. More than 400 mycotoxins are known to exist in nature. Contamination of foods and feeds with mycotoxins is a worldwide serious problem. The most important mycotoxins in terms of toxic effect on both humans and animals are aflatoxins (AFs), citrinin (CIT), cyclopiazonic acid (CPA), fumonisins (FBs), moniliformin (MON), ochratoxin A (OTA), deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), patulin (PAT), zearalenone (ZEA), and ustiloxins. These mycotoxins have several adverse impacts on consumers, such as loss of human and animal lives, health-care and veterinary care costs, contaminated food and feed disposal costs, and huge investment in research and management of the mycotoxin problem. The mycotoxins induce diverse biological effects,

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which have been characterized on animals and humans. These toxic effects of mycotoxins include cytotoxic, carcinogenic, immune suppressive, nephrotoxic neurotoxic, mutagenic and estrogenic effects, etc.

29.1 Introduction

Rice, wheat, and maize together accounted for 89% of all cereal production worldwide and 43% of all food calories in 2012 (FAO 2013). Rice is a common food crop and used as a dietary staple for the people in many parts of the world. It is known to be attacked by 56 fungal pathogens (Ou 1985), of which 41 are reported to be seed-borne (Richardson 1979, 1981). Wheat provides about 20% of the world's food calories, and it is a staple food for nearly 40% of the world's population. Seed-borne fungi including the genera of *Tilletia, Ustilago, Bipolaris, Fusarium, Alternaria, Drechslera, Stemphylium, Curvularia, Cladosporium, Rhizopus, Aspergillus,* and *Penicillium* have been convincingly reported in wheat throughout the world (Nirenberg et al. 1994; Rehman et al. 2011; Suproniene et al. 2011). Maize is susceptible to as many as 112 diseases, and among them, more than 70 are seed-borne on a global basis (USDA 1996), and about 35 of these diseases are recorded in India (Sharma and Lal 1998). The disease spectrum varies in different agroclimatic zones, but the more serious diseases are leaf blights, downy mildews, stalk rots, and rusts. In developing countries with poor farming practices, seed-borne pathogens are among the major constraints.

Seed is the basic unit of crop production and has greater contribution to environmental and cultural factors. Virtually, 90% of all the world's food crops are grown from seeds (Schwinn 1994) and are also used in national and international trade. In several breeding programs, germplasm is also distributed in the form of seeds. However, many plant pathogens can be seed-transmitted, and plant pathogens are introduced into new areas through seed, as well as seed serves as the source of survival of the pathogen between growing seasons. The seeds carry a number of fungal pathogens, which can be divided as two groups: the "field fungi" and the "storage fungi" (Atanda et al. 2011). The global losses due to seed-borne diseases are estimated at 12% of potential production (Agarwal and Sinclair 1987). If untreated seeds are grown in the field, seed-borne pathogens can reduce the crop yield up to 15–90% (Zafar et al. 2014). Stored products are a man-made ecosystem in which quality and nutritive changes occur because of interactions between physical, chemical, and biological factors. Fungal spoilage and mycotoxin contamination are of major concerns (Chulze 2010). The original source of fungi is in the field. Storage fungi include numerous species of *Aspergillus, Fusarium,* and *Penicillium* (CAST 2003). The growth of fungi in storage is governed by composition of nutrients in the grain, moisture, and temperature conditions, biotic factors like competition, or the presence of storage insects (Atanda et al. 2011). Storage fungi are much more frequent in lots infested by stored product insects because insects generate moisture and distribute fungal spores in the commodity. Storage fungi require a relative humidity of at least

65% (a_w = 0.65), which is equivalent to an equilibrium moisture content of 13% in cereal grain. They grow at temperatures between 10 °C and 40 °C. Every fungal species has its own optimum climatic requirements for the growth and development (Multon 1988). During storage, high temperatures and relative humidity can also contribute to the deterioration of the seeds due to lipid peroxidation (Nakagawa and Rosolem 2011). High relative humidity promotes the re-initiation of metabolic activity in the embryo, whereas high temperatures increase respiratory activity, which depletes the reserve material. In addition, these conditions may favor fungal and insect activity, thus reducing seed quality (Christensen and Kaufmann 1965). Storage fungi cause discoloration and rotting, affecting viability as well as commercial and nutritional value of seeds because of increased fatty acid content, oil rancidity, and heating of the seed mass, which increases the respiratory rate and accelerates deterioration (Nóbrega and Suassuna 2004; Bellettini et al. 2005; Borém et al. 2006; Santos et al. 2013). *Aspergillus flavus* is generally the most damaging species in cereals and nuts (Hocking and Banks 1991; Santos et al. 2016).

The word mycotoxin comes from words "*mykes*^{G}" and "*toxicum*^{L}" meaning fungus and toxin or poison, respectively (Hundley 2001; Chu 2002, 2006). Mycotoxin is defined as "natural product produced by fungi that elicit a toxic response when introduced in low concentration to vertebrates by a natural route." Approximately 300–400 mycotoxins are known to exist in nature (Huff et al. 1992), but there are some others that are being discovered and still awaiting classification. Mycotoxins have a wide range of chemical structures, and they have different physiological activities ranging from neutral to toxic potentials (Richard 2007; Njobeh et al. 2010). Mycotoxins are secondary metabolites produced by various fungi growing as contaminants on storage crops in particular cereals, nuts, and fruits. These infected crops used for food and feeds are toxic and have many adverse effects on health of human beings and animals. During end of the first millennium, "St Anthony's fire" was one of the well-known recognized diseases caused by mycotoxins in Europe. This was due to ergot alkaloids produced by the mould *Claviceps purpurea* in rye. The epileptic fits and severe burning sensation experienced led numerous pilgrims to the shrine of St Anthony in France in hope of a relief (Bove 1970). In 1918, Ergotamine was first isolated and marketed as a safe and more authentic form than the ergot extracts. In the 1930s, some well-planned trials showed it to be effective in migraine headache (Hart 1999). The death of 100,000 turkey poults and other poultry in the UK just before Christmas in 1960 was eventually traced to a toxic contaminant later called aflatoxin that was present in the groundnut meal forming part of their diet. This incident illustrated the potential threat posed by mycotoxins and ushered in the modern era of mycotoxin studies (Klich 2002; Papp et al. 2002; Kuhn and Ghannoum 2003). Historically, the first case of linking molds to an aggravation of asthma appeared in 1698; the patient had experienced an "asthmatic attack" because he came into contact with fermenting wine (Chowdhary et al. 2014). However, others are known to be pathogenic, causing several diseases in plant and animal species (Pitt and Hocking 2009). Although these are useful in the food and beverage industries as well as in the production of antibiotics and organic acids (Blackwell et al. 2009), such negative impact of fungi on agricultural production

either as pathogens or as mycotoxin producers is usually associated with severe yield loss, depletion of nutritive value, and health-related problems (Blackwell et al. 2009). There is an increased multitude of fungal diseases in certain conditions with severe economic implications on a worldwide scale (Annaisie et al. 2002). Particularly, *Fusarium* head blight disease of cereals is increasing, i.e., resulting in the significant loss of grain yield (Demeke 2005). Mycotoxin consumptions may have implications on consumers, such as loss of human and animal lives, health-care and veterinary care costs, contaminated food and feed disposal costs, and investment in research and management of the mycotoxin problem. Mycotoxins are able to induce powerful and diverse biological effects. Diverse actions of mycotoxins have been characterized on animals and humans to include cytotoxic, carcinogenic, immunosuppressive, nephrotoxic, neurotoxic, mutagenic, and estrogenic effects (Krska et al. 2007). Type of toxicity depends on the type of toxin, amount, and duration of exposure. In the African continent, a cumulative effect between mycotoxins and some important diseases such as tuberculosis, malaria, Kwashiorkor, and AIDS/HIV has been suggested (Turner et al. 2003; Gong et al. 2003, 2004). In addition, they have been in relevance to the etiology of several diseases in humans and animals. Now-a-days, mycotoxins are recognized as causal factors for primary liver cancer, ergotism, and alimentary toxic aleukia (Shephard et al. 2007). They are able to reduce livestock productivity via growth depression due to immunosuppression, increased secondary bacterial infections, interference with reproductive capacity, and, in severe cases, sometimes mortality. Different structural diversities of mycotoxins, their effects, and symptoms they may generate vary significantly (Njobeh et al. 2010). Furthermore, there are equal variations of such toxicities among the different animal species and even among individuals of the same races or breeds (Kuiper-Goodman 2004).

The naturally toxigenic fungal flora, existing in conjunction with food production, is dominated by most dominant genera, namely, *Aspergillus, Fusarium,* and *Penicillium* and, to a lesser extent, the *Alternaria, Claviceps,* and *Stachybotrys* (Tuite and Forester 1979; Pitt and Hocking 1997). The economically important species of fungi producing significant mycotoxins as: fumonisins (FBs), aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), and nivalenol (NIV). T-2 toxin (T-2), cyclopiazonic acid (CPA), patulin (PAT), and zearalenone (ZEA) are given (Pitt and Hocking 2009) in Table 29.1. Food-borne mycotoxins such as aflatoxins, zearalenone, and fumonisins are likely to be of greatest importance for human health in tropical developing countries. The LC-MS/MS method was used to screen more than 80 feed and feed raw materials which resulted in the occurrence of 340 mycotoxins and other secondary metabolites in them (Streit et al. 2013). In the surveyed samples sourced 2004 till 2011 worldwide, DON was the most dominant with 55% of the samples tested positive, followed by FBs (54%), ZEA (36%), AFs (27%), and OTA (25%) (Schatzmayr and Streit 2013). Estimates suggest that >600,000 people die of liver cancer worldwide each year with a majority of cases occurring in China, Southeast Asia, and sub-Saharan Africa. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) were represented by 360 million and 170 million people, respectively, worldwide (Michielsen et al.

Table 29.1 Mycotoxins: types, classification, food commodity, action level, mode of action, and pathological effects

S no.	Mycotoxins	Type (IARC classification)	Fungal species	Food commodity	Action level	Mode of action	Pathological effects
1.	**Aflatoxins (6T)**	AFB_1(1), AFB_2(1), AFG_1(1), AFG_2(1), AFM_1(2B) & AFM_2(2B)	*Aspergillus flavus, A. nomius, A. parasiticus, A. arachidicola, A. bombycis, A. pseudotamarii, A. minisclerotigenes, A. rambellii, A. ochraceoroseus, Emericell astellata, E. venezuelensis, E. olivicola* (CAST 2003)	Maize, wheat, rice, spices, sorghum, groundnut, tree nut, almonds, milk, oilseeds, dried fruit, cheese, eggs, and meat (D'Mello 2003)	AFB_1, AFB_2 = <20 ppb AFM_1 & AFM_2 < 0.5 ppb (Wild and Gong 2010)	Cytochrome P450 enzymes convert aflatoxins to the reactive 8,9-epoxide form, which is capable of binding to both DNA and proteins. Aflatoxin B_1-DNA adducts can result in GC to TA transversions (Raj et al. 1986)	Carcinogenic, mutagenic, teratogenic, hepatotoxic, nephrotoxic, immunosuppressive, hemorrhage of the intestinal tract and kidney and liver disease (Wild and Gong 2010)
2	**Citrinin**	CIT (3)	*Penicillium citrinum, P. camemberti, Aspergillus terreus, A. oryzae, A. niveus, Monascus ruber,* and *Monascus purpureus* (Hetherington and Raistrick 1931; Ciegler et al. 1977; Bragulat et al. 2008)	Wheat, oats, rye, corn, barley, and rice (Bragulat et al. 2008)	<100 ppb (Chung et al. 2009)	To depress RNA synthesis in murine kidneys (Sansing et al. 1976)	Nephrotoxin, cough, shortness of breath, headache, nausea, vomiting, central nervous system depression, narcosis (Thrasher and Crawley 2009)

(continued)

Table 29.1 (continued)

S no.	Mycotoxins	Type (IARC classification)	Fungal species	Food commodity	Action level	Mode of action	Pathological effects
3	**Cyclopiazonic acid**	CPA	*Penicillium cyclopium, P. patulum, P. viridicatum, P. puberulum, P. crustosum, P. camembertii, Aspergillus versicolor, A. oryzae, A. tamarii,* and *A. flavus* (D'Mello 2003)	Peanuts, sunflower seeds, millet, and Kodo (D'Mello 2003)	<10 ppm (Lomax et al. 1984)	Inhibits the Ca^{2+}-stimulated ATPase and Ca^{2+} transport activity of the sarcoplasmic reticulum (Seidler et al. 1989)	Behavioral: Somnolence (general depressed activity). Liver: Hepatitis (hepatocellular necrosis), diffuse. Kidney, ureter, bladder: changes in tubules (including acute renal failure, acute tubular necrosis) (Purchase 1971; Nishie et al. 1987; Moncoq et al. 2007)
4	**Ergot alkaloids (2T)**	Ergotamine and ergovaline	*Claviceps africana, C. purpurea, C. fusiformis, C. paspali,* and *Neotyphodium coenophialum* (D'Mello 2003)	Wheat, rye, hay, barley, millet, oats, sorghum, and triticale (D'Mello 2003)	<1 ppb (Anonymous 2012)	The serotonin syndrome can be caused by binding of dihydroergotamine to serotonin receptors in the dorsal horn of the spinal cord, which is the site of neuropathological changes in convulsive ergotism (Eadie 2003)	Gangrenous form: vasoconstrictive activity (edema of the legs, paresthesia, gangrene at the tendons); convulsive form: gastrointestinal symptoms (nausea, vomiting) effects on the central nervous system (drowsiness, ataxia, convulsions, blindness, and paralysis) (Wiese 1987; Karow 1997; Bennett and Klich 2003)

5	**Fumonisins (6T)**	FB_1(2B), FB_2(2B), FB_3(2B), FB_4(2B), FA_1(2B) & FA_2(2B)	*Fusarium anthophilum, F. moniliforme, F. dlamini, F. napiforme, F. proliferatum, F. nygamai, F. verticillioides Alternaria alternata* (Picot et al. 2010; Stepien 2014)	Maize, maize-based products, corn-based products, sorghum, asparagus, rice, and milk (D'Mello 2003)	<2000 ppb (Wild and Gong 2010)	Interfering with sphingolipid metabolism (Merrill et al. 2001)	Hepatotoxic, cerebral edema, necrosis, and immunotoxic (Wild and Gong 2010)
6	**Moniliformin (C_4HO_3Na)**	MON	*F. moniliforme var. subglutinans, F. proliferatum, F. anthophilum, F. graminearum, F. avenaceum, F. acuminatum, F. concolor, F. equiseti, F. oxysporum, F. semitectum,* and *F. fusarioides* (*F. chlamydosporum*), and *Sporotrichiella* (Scott et al. 1987)	Oats, wheat, corn, rye, triticale, and banana (D'Mello 2003)	<100 ppm (Sharma et al. 2008)	Inhibitor of several thiamine pyrophosphate-depending enzymes with pyruvate dehydrogenase as best studied resulting in inhibition of gluconeogenesis (Burka et al. 1982; Pirrung et al. 1996)	Kashin-Beck disease (KBD), chondrocyte necrosis, apoptosis (Zhao 2002), and DNA damage (Cao et al. 1995). Selenium deficiency and iodine deficiency (Li et al. 2008)

(continued)

Table 29.1 (continued)

S no.	Mycotoxins	Type (IARC classification)	Fungal species	Food commodity	Action level	Mode of action	Pathological effects
7	**Ochratoxin**	OTA(2B), OTB(2B), OTC(2B)	*Aspergillus ochraceus, A. alutaceus, A. alliaceus, A. auricomus, A. glaucus, A. niger, A. carbonarius, A. melleus, A. albertensis, A. citricus, A. flocculosus, A. fonsecaeus, A. lanosus, A. ostianus, A. petrakii, A. sulphureus, A. pseudoelengans, A. roseoglobulosus, A. sclerotiarum, A. steynii, A. westerdijkiae, Neopetromyces muricatus, Penicillium viridicatum, P. verrucosum, P. cyclopium*, and *P. carbonarius* (D'Mello 2003)	Cereals, dried vine fruit, wine coffee, oats, spices, rye, raisins, and grape juice (Halasz et al. 2009)	OTA = <5 ppb (Murphy et al. 2006)	Inhibits the enzyme involved in the synthesis of the phenylalanine tRNA complex (Bunge et al. 1979; Marquardt and Frohlich 1992). In addition, it inhibits mitochondrial ATP production (Meisner and Meisner 1981)	Carcinogenic, mutagenic, teratogenic, hepatotoxic, nephrotoxic, immuno-depressants, and inhibition of protein synthesis (Thrasher and Crawley 2009)

8	**Patulin**	PAT (3)	*Aspergillus clavatus, A. longivesica, A. terreus, P. expensum, P. griseofulvum, Byssochlamys* sp. (D'Mello 2003)	Apples, apple juice, cherries, cereal grains, grapes, pears, and blueberry (Tribst et al. 2009)	<50 ppb (Murphy et al. 2006)	Reacts with sulfhydryl groups and to induce oxidative damage (Liu et al. 2007). Also reduces the cytokine secretion of IFN-γ and IL-4 by human macrophages (Wichmann et al. 2002; Luft et al. 2008)	Immuno-depressant, pulmonary and cerebral edema, nausea, gastritis, paralysis, convulsions, capillary damage, and carcinogenic (Thrasher and Crawley 2009)
9	**Sterigmatocystin**	ST(2B)	*Aspergillus nidulans, A. tamarii, A. ochraceoroseus,* and species of *Bipolaris, Chaetomium, Farrowia,* and *Monocillium* (Barnes et al. 1994; Klich et al. 2000)	Wheat, maize, hard cheese, and green coffee beans (Versilovskis and De Saeger 2010)	<5 ppb (Veršilovskis and Bartkevičs 2012)	The binding to the same N_7 guanine adduct of DNA molecules just like aflatoxin (Versilovskis and De Saeger 2010)	Diffuse hepatocellular necrosis, hepatitis, fatty liver degeneration, liver tumors, carcinogenic to humans (Thrasher and Crawley 2009).

(continued)

Table 29.1 (continued)

S no.	Mycotoxins	Type (IARC classification)	Fungal species	Food commodity	Action level	Mode of action	Pathological effects
10	**Trichothecenes**	T-2 (3) and HT-2 toxin (3), diacetoxyscirpenol, Neosolaniol, nivalenol (NIV) (3), deoxynevalenol, 3-acetyl DON (3), 1,5-acetyl DON, fusarenon X (3)	*Fusarium sporotrichioides, F. poae, F. acuminatum, F. culmorum, F. equiseti, F. graminearum, F. cerealis, F. moniliforme, F. myrothecium, F. lunulosporum, Cephalosporium* sp. (D'Mello 2003)	Cereal and cereal based products (D'Mello 2003)	T-2 = <100 ppb, DON = <1000 ppb (Murphy et al. 2006) NIV = <0.2 ppm	Acting both on the cell immune system and on the number of macrophages, lymphocytes, and erythrocytes. T-2 and deoxynivalenol (DON) are known to inhibit protein synthesis and cause cell death in various parts of the body (Upadhaya et al. 2010)	Immuno-depressants, mutagenic, gastrointestinal hemorrhaging, and neurotoxic (Thrasher and Crawley 2009)

11	**Zearalenone**	α-ZON (3) and β-ZON (3)	*Fusarium graminearum, F. culmorum, F. crookwellense, F. equiseti*, and *F. sporotrichioides* (D'Mello 2003)	Barley, oats, wheat, rice, sorghum, sesame, soybeans and cereal-based products (D'Mello 2003)	<1 ppm (Abdelhamid 1990)	Binds to estrogen receptors (Zinedine et al. 2007). Decrease in the amounts of luteinizing hormone (LH) and progesterone produced affecting the morphology of uterine tissues, decrease in milk production, feminization of young males due to decreased testosterone production (Guerre et al. 2000)	Estrogenic activity (infertility, vulval edema, vaginal prolapsed, mammary hypertrophy in females, feminization of males (Thrasher and Crawley 2009)
12	**Ustiloxins**	UA, UB, UC, UD, and ustilaginoidins	*Ustilaginoidea virens*	Rice	–	Inhibitor of tubulin polymerization and mitosis of human tumor cell line (Koiso et al. 1994; Li et al. 1995)	lupinosis in mice and toxicosis of cattle (Nakamura et al. 1994)

Note: 1 μg/Kg = 1 ppb, 1 ppm = 1000 ppb, 1 mg/Kg = 1 ppm

2005). Environmental, socioeconomic, and poor agricultural practices favor fungal growth and also contribute to increased contamination levels of these mycotoxins in food and feed commodities. An attempt is also made to review measures adopted to reduce mycotoxin and the inherent problems like health intimations and legislation associated with such contaminations. Several factors influence regulatory levels for recommended mycotoxin daily intake limits in human and animals which are based on estimated results from animal studies. Action levels and acceptable levels are set up by joint meetings of some governing bodies including the FAO, WHO, IARC, JECFA, NNT, PROMEC, EU, etc. after inspection of expert reports on mycotoxins (Njobeh et al. 2009).

29.2 Aflatoxins (AFs)

Aflatoxins are among the most important and the most studied of all mycotoxins since their discovery in the 1960s by a group of British researchers investigating mycotoxicosis outbreaks in poultry (Richard 2008). Since their early discovery, studies on AF still dominate mycotoxin research probably because they are the most flagrant of all mycotoxins (Njobeh et al. 2010). Major threats are in the tropics and desert environments where *A. flavus* and *A. parasiticus* are more dominant, affecting mainly cereals, nuts, and by-products. *Aspergillus flavus* and *A. parasiticus* are major AF producers, and some strains of *A. tamari, A. ochraceoroseus, A. rambellii,* and *A. nomius* as well as other fungal species including *Emericella venezuelensis* and *E. astellata* have been found in food and feed materials (Klich et al. 2000; Vargas et al. 2007). Aflatoxins may contaminate many crops including corn, peanuts, cottonseed, Brazil nuts, pistachios, spices, copra, and figs in hot and humid regions of the world. This mycotoxin occurs in several forms designated, i.e., aflatoxin B1, B2, G1, G2, M1, and M2. "B" and "G" mean to the blue or green fluorescence of the toxin observed under ultraviolet irradiation. M_1 and M2 are derivative metabolic products of B1 and B2 produced in milk from lactating humans and animals due to contaminated food or feeds. In the studies of staple foods, which were contaminated with 5000 ppb or above of aflatoxins were found associated with fatality although daily consumption of foods with >1000 ppb was linked to aflatoxicosis in Kenya and India. A risk of fatality can be estimated to be >1 mg/day intake of total aflatoxins or in excess of 20 μg/kg body wt/day in adults, based on the observed levels of contamination (Krishnamachari et al. 1975; Ngindu et al. 1982). Aflatoxins are immunosuppressive, mutagenic, carcinogenic, and teratogenic in nature.

Immunotoxicity seems to be an effect of human AFB_1 exposure in which lymphocyte immune functions were suppressed by this mycotoxin. Immunoglobulin a response to few vaccine challenges was suppressed in Gambian children with detectable AFB_1-albumin adducts (Turner et al. 2003). Aflatoxins are a Group-1 carcinogenic toxins (IARC 2002), liver and kidney being the most susceptible organs (Devegowda et al. 1998). The toxicogenic effects result from ingestion of food contaminated with AFs ranging from acute liver disease to more chronic one that causes cancer. Aflatoxins are also the persistence of hepatitis B antigen (HB

Ag), which reflects a defective immune response that is associated with hepatoma caused by aflatoxin intoxication. Cerebral edema is characterized as Reye's syndrome and convoyed by fatty acid disintegration of the liver, kidneys, myocardium, and fibers of the striated muscles due to AF intoxication. It has also been postulated that aflatoxins directed primarily many clinical features of Kwashiorkor toward the liver (Hendrickse 1985). Investigations have found a strong relationship between hepatic impairment and aflatoxins among Kwashiorkor children. Certain human diseases such as Reye's syndrome, Kwashiorkor, acute hepatitis, Indian childhood cirrhosis, chronic gastritis, and some occupational diseases have been associated with foods contaminated with AFs. Exacerbated protein calorie malnutrition and resulted growth suppression; suppressed immune function are likely correlated to AFB_1 infection (Wild and Hall 1996; Sibanda et al. 1997).

Greater reductions of aflatoxins have been found in roasted peanuts than in boiled peanuts (Njapau et al. 1998). Approximately 50% detectable aflatoxin was reduced in the fermentation of wheat flour dough, while baking of the dough resulted ranging from 0% to 25% reductions (Scott 1991). Traditional nonalkaline toasting and boiling processes to produce pinole from aflatoxin-contaminated corn were reported to significantly reduce detectable aflatoxin (Mendez-Albores et al. 2004). Alkaline treatment of contaminated corn prior to frying resulted in very low levels of chemically detectable aflatoxin at 121 °C (Camoou-Arriola and Price 1989). Nixtamalization processing of corn resulted in significant reductions of aflatoxin (Torres et al. 2001). Chlorophyllin and oltipraz have been protecting against human AFB_1 toxicity for carcinogenesis (Kensler et al. 2004).

29.2.1 Mechanisms of Action

AFB_1 is metabolized by the cytochrome P450 enzyme system in the liver to produce the major carcinogenic metabolite AFB1-8, 9-epoxide (AFBO), or to less mutagenic forms such as AFM_1, Q1, or P1 (Shimada and Guengerich 1989; Crespi et al. 1991). AFBO readily binds to cellular proteins and DNA, to form adducts. Aflatoxin B_1 induces cancer via metabolic cytochrome P450 enzyme system, specifically CYP3A4, CYP3A5, and CYP1A2 (Wang et al. 1991) to exo-8, 9-epoxide, which can form adduct with DNA leading to guanine nucleotide substitutions (Lillerberg et al. 1992). AFBO adducts, such as with N_7-guanine, can lead to gene mutations and cancer. Mutation is suspected to occur in the human p53 tumor suppression gene at codon 249 (AGG) (Eaton and Gallagher 2004). AFBO induces conversions from G to T at the third nucleotide of the codon, making it a mutational "hotspot." This mutation has been shown with high frequency among patients with hepatocellular carcinomas in areas of high-risk AF exposure (IARC 1993b, c). The AFB_1-N_7-guanine adduct is excreted in the urine of those infected and serves as evidence that humans have the necessary biochemical pathways for carcinogenesis (IARC 1993b, c). Aflatoxin enters the cell and metabolizes in the endoplasmic reticulum to hydroxylated metabolites that are further metabolized to glucuronide and sulfate conjugates or is oxidized to the reactive epoxide that undergoes hydrolysis and can bind to proteins

resulting in cytotoxicity (Wang et al. 1991). AFs caused hazardous effects on human health, including hepatocellular carcinoma by aflatoxin B_1. Aflatoxin-B_1 is converted into aflatoxin B1-8,9 exo-epoxide, which is in turn converted into 8,9-dihydroxy-8-(N7) guanyl-9-hydroxy. This metabolized product is mutagenic and carcinogenic. AFB_1 produced synergistically with hepatitis B virus in causing hepatocellular carcinoma disease (Kew 2013).

29.3 Citrinin (CIT)

Citrinin is a polyketide mycotoxin ($C_{13}H_{14}O_5$) with a molecular weight of 250.25 g/mol (Steyn 1998; Xu et al. 2006). CIT has a common structural similarity with OTA and co-occurs in cereals with it. Approximately 26 fungal species can synthesize CIT (Dirheimer 1998), mainly *Penicillium citrinum* (D'Mello 2003), and most often 14 species of *Penicillium* and three species of *Aspergillus* (Kurata 1990). Citrinin was the earliest mycotoxin discovered in 1931 by Hetherington and Raistrick, which was illustrated from several strains of *P. citrinum* and further confirmed by Xu et al. (2006). Citrinin has been reported as natural occurrence in cereal grains such as wheat, barley, oats, rice, corn, and its products such as agricultural commodities, foods, and feedstuffs as well as biological fluids in mammals (CAST 2003).

Citrinin has antibiotic, bacteriostatic, antifungal, and antiprotozoal properties. It is also known as a hepato-nephrotoxin in a wide range of species. CIT affects primarily the kidney of poultry. Regarding its relative toxicity, CIT appears to be less toxic to poultry than either nephrotoxic oosporein or ochratoxin-A. High levels of citrinin may affect the liver in addition to the kidney. Clinical symptoms of toxicity by citrinin are increased water consumption and diarrhea in poultry. These symptoms have been shown at low levels ranging from 130 ppm to 260 ppm dietary citrinin. Due to altered function and degenerative processes of the renal tubules, there appears to be diarrhea and increased urine excretion. When CIT and OCA co-occur in grain and then fed to animals, there can be an acceleration of the effects because of the similarity in both toxins (Bilgrami et al. 1988; Berndt 1990).

29.3.1 Mechanisms of Action

CIT toxicity and genotoxicity are the consequence of oxidative stress or increased permeability of mitochondrial membranes. CIT requires complex cellular biotransformation to exert mutagenicity (Da Lozzo et al. 1998). CIT treatment induced nucleus crenation, loss of nucleolus, depletion of cytoplasmic organelles, mitochondrial pleomorphism, nuclear fragmentation, uniform folding of cell membrane, and cytoplasmic vacuolation in proximal convoluted tubules (Kumar et al. 2007). Citrinin has been found to induce loss of selective membrane permeability, leading to cell disruption and a likely consequence of apoptotic cell death (Ansari et al. 1991). CIT induces pyruvate and keto-glutarate decarboxylase and loss of cellular respiration control and eventual cell death in guinea pigs (Thiel 1978).

29.4 Cyclopiazonic Acid (CPA)

Cyclopiazonic acid (α-cyclopiazonic acid, CPA; $C_{20}H_{20}N_2O_3$) is an indole-tetramic acid mycotoxin produced by many species of *Aspergillus* and *Penicillium*. The compound was originally isolated from *Penicillium cyclopium* and hence the name of mycotoxin is cyclopiazonic acid. Other organisms include *P. patulum, P. viridicatum, P. puberulum, P. crustosum, P. camembertii, Aspergillus versicolor, A. oryzae, A. tamarii,* and *A. flavus* (Holzapfel 1968; Hermansen et al. 1984; Frisvad 1989; Burdock and Flamm 2000). Besides colonizing various grains and seeds, these molds can grow on many food substrates, such as cheese and meat products. This compound has also been found in peanuts, sunflower seeds, and Kodo millet likely as naturally occurring. CPA gathers in the skeletal muscle of animals and humans, and acquaintance may occur through ingestion on the contaminated muscle tissue. Toxic sign in animals, depending upon the species, includes gastrointestinal necrosis and inflammation, hepatitis, kidney lesions and incoordination due to effects on the muscle tissue. The importance of CPA compound in immunosuppression has been studied with little significance on this system (Abbas et al. 2008; Njobeh et al. 2009).

29.4.1 Mechanisms of Action

CPA has the ability to alter normal intracellular Ca^{++} flux through the specific inhibition of sarcoplasmic endoplasmic reticulum calcium-dependent ATPase (SERCA) essential for calcium uptake as in the muscle contraction-relaxation cycle, which results in increased muscle contraction (Goeger et al. 1988; Riley et al. 1992). SERCA serves a housekeeping function, maintaining high calcium in the endoplasmic reticulum and low cytosolic calcium. This calcium gradient is vital for the cell and controls proliferation, differentiation, and cell death. A recent study showed that CPA inhibits the calcium pump by blocking the calcium entry channel and immobilizing a subset of four transmembrane helices of the ATPase (Moncoq et al. 2007). Along with the definite inhibition of ATPase activity, CPA induced various pathological lesions in test animals (Burdock and Flamm 2000).

29.5 Ergot Alkaloids

Ergotism is the first best historically documented example of mycotoxicosis (Fajardo et al. 1995). Ergot alkaloids, mostly *Claviceps purpurea,* are produced in rye and other grains such as barley, corn, millet, oats, rice, sorghum, and wheat (Bennett and Bentley 1999). Major ergot alkaloids comprising lysergic acid derivatives as ergocristine and ergotamine and other derivatives such as ergosine, ergocornine, and ergometrine occur in contaminated cereal grains. A periodic outbreak of St Anthony's fire/ergotism caused ergots in Europe during the middle ages (Van Rensburg 1977). In the twentieth century, the famous hallucinogen ergot alkaloid, i.e., lysergic acid diethylamide (LSD), was discovered at the Sandoz Laboratories in

Basel, Switzerland. A chemist named Hofmann combined different amines in peptide linkage with lysergic acid to produce the first ergobasine semisynthetic ergot alkaloid (Hofmann 1972). *Claviceps purpurea* has been recorded in at least 17 species of grasses (Loveless 1971). Matossian (1981) proposed that the "slow nervous fever," described by physician Jon Huxham in the eighteenth century, may be another example of human ergotism. Symptoms of ergotism include gangrene, abortion, convulsions, suppression of lactation, hypersensitivity, and ataxia in animals (Lorenz 1979). The prehistoric Chinese and Europeans used ergot alkaloid compounds to reduce bleeding after childbirth and to persuade abortions when desirable. Numerous valuable medications have been industrialized to treat bleeding, muscle spasms, and migraine headaches. Two ergot alkaloid compounds have shown amazing efficiency in treating migraine headaches. Research is ongoing to find more and diverse medical uses for ergot alkaloids (Wiese 1987).

29.5.1 Mechanisms of Action

Ergot alkaloid amide and peptide derivatives have a variety of physiological effects, including serotonin and dopamine-receptor agonists and competitor, vasoconstrictors, neurotoxins, and hallucinogens (Hart 1999).

29.6 Fumonisins (FBs)

Fumonisins are polyketide mycotoxins produced predominantly by *Fusarium verticillioides* and *F. proliferatum*, and at very low levels by *Alternaria* in black end stem rot in tomato (Chen et al. 1992), asparagus, and garlic (Seefelder et al. 2002). The fumonisins occur mostly in corn grains, but several researches have reported its occurrence in other grains, for example, rice (Park et al. 2005) and wheat and oat (Mallmann et al. 2001). Structurally, 16 fumonisins have been isolated and characterized: FB1, FB2, FB3, FB4, A1, A2, A3, AK1, C1, C3, C4, P1, P2, P3, PH1A, and PH1B. However, FB1 and FB2 are the most important, and up to 70% of these fumonisins occurs in naturally contaminated foods and feeds (Seo et al. 2001; Niderkon et al. 2009). FBs were first described and characterized in 1988 (Bezuidenhout et al. 1988). Their structures are based on a long hydroxylated hydrocarbon chains containing methyl and either amino (B1–B2) or acetyl amino groups (A1–A2) (Powell and Plattner 1994). FB1 is responsible for equine leukoencephalomalacia (ELEM) and pulmonary edema of horses and swine, respectively. Hepatotoxicity and nephrotoxicity have also been reported in linking with fumonisin intoxication. The fumonisins are potent human carcinogens and responsible for increased prevalence of esophageal cancers in South Africa and China (IARC 1993b, c). FBs have been associated with an outbreak in humans characterized by abdominal pain and diarrhea in 27 villages on the Deccan Plateau in India in 1995 due to consumption of contaminated sorghum and maize (Bhat et al. 1997). FBs are known to interrupt sphingolipid concentrations and synthesis, which may elucidate

the different etiologies of fumonisin toxicity in livestock and humans (Merrill et al. 1996). Wet milling of contaminated corn partitioned fumonisins to all fractions (gluten > fiber > steep water > germ) except for starch fraction (Bennett et al. 1996). Dry milling of contaminated corn partitioned fumonisin to all fractions, with the bran containing the highest levels (bran > germ > flour > flaking grits) of white, yellow, and blue corn (Katta et al. 1997). Jackson et al. (1996) found that FB was least stable at pH 4 and high temperature processing may be effective against FBs in some cases. Alkaline processing has been revealed to be effective in reducing FB levels and activity.

FB1 is reported to be the most toxic endorsing tumor in rats (Gelderblom et al. 1998). FBs mostly affect nonruminants, i.e., horses, pigs, poultry, etc., and ruminants seeming to be much less sensitive to this type of contamination (Yiannikouris and Jouany 2002). However, *Fusarium*-contaminated wheat fed to dairy cows led to increased crude protein degradation and a lower molar percentage of propionate in the rumen (Tiemann and Dänicke 2007). FBs cause deep lesions in the liver, gastrointestinal tract, nervous system, and lungs. Acute doses of FBs in pigs may inhibit the activity of pulmonary macrophages responsible for the eradication of pathogens, prominent to pulmonary edema (Harrison et al. 1990). In horses, contamination is exhibited as severe neurological lesions leading to locomotive problems and ataxia (Yiannikouris and Jouany 2002). The highest plant populations had higher incidence of *Fusarium* infection (>24%) in the kernel and higher fungal ear rot severity (>43%) than plots with lower plant densities. Natural existence of mycotoxins was always significantly higher in crops with a higher plant density (Marín et al. 2012).

29.6.1 Mechanisms of Action

FB1 is a carcinogen to animals. There are different potential mechanisms by which it may exert its effects (Stockmann-Juvala et al. 2008). FB1 induces micronuclei and chromosomal aberrations in primary hepatocytes and Hep-G2 cells (Merrill et al. 2001; Ehrlich et al. 2002; IARC 2002). FB1 increases oxidative DNA damage and increased DNA strand breaks and malondialdehyde adducts in the liver and kidney of rat (Domijan et al. 2006) and lipid peroxidation (Abel and Gelderblom 1998) in vivo. The toxic action of FBs inhibits the synthesis of ceramides from sphinganine, blocking the biosynthesis of sphingolipids (Yoo et al. 1996; Merrill et al. 1997). Tricarballylic acid of FB1 hydrolyis-made aminopentol isomers has been suggested to cause toxic effects because of the structural similarity to sphingoid bases (Humpf et al. 1998). Complex sphingolipid biosynthesis inhibition disrupts several cell functions and signaling pathways, including apoptosis and mitosis, therefore possibly contributing to carcinogenesis through a transformed balance of cell death and replication (Stockmann-Juvala et al. 2008). Animal studies have demonstrated that FBs exposure can cause neural tube defects (NTDs) (Gelineau-van Waes et al. 2005). It is of concern that FB could cause similar effects in humans (Marasas et al. 2004). FB was reported to induce liver and kidney tumors in rodents and are classified as Group 2B "possibly carcinogenic to humans," with ecological

studies implying a possible link to increased esophageal cancer (Wild and Gong 2010). Fumonisin toxin causes "crazy horse disease" or leukoencephalomalacia, a liquefaction of the brain. Symptoms include blindness, head butting and pressing, constant circling, and ataxia, followed by death. Chronic low-level exposure in humans causes esophageal cancer and neural tube defect disease (Yiannikouris and Jouany 2002).

29.7 Moniliformin (MON)

Moniliformin is the Na/K salt of 1-hydroxycyclobut-1-ene-3,4-dione (Springer et al. 1974; Scharf et al. 1978) and was discovered as a mycotoxin from *Fusarium moniliforme* by Cole et al. (1973). *Fusarium* species that also produce moniliformin are *F. moniliforme* var. *subglutinans*, *F. proliferatum, F. anthophilum, F. graminearum, F. avenaceum, F. acuminatum, F. concolor, F. equiseti, F. oxysporum, F. semitectum, F. fusarioides, F. sporotrichioides,* and *F. culmorum* (Cole and Cox 1981; Rabie et al. 1982; Marasas et al. 1986). Kashin-Beck disease (KBD) is an endemic chronic deformative osteoarthropathy (Cao et al. 2007) affecting a huge proportion of population in China. The pathological alterations are seen in articular cartilages of main joints, for example, knee and elbow joints (Cao et al. 2008). KBD patients show chondrocyte necrosis in the hypertrophied layer of the cartilage adjacent with cell cluster formation (Li et al. 2008). The etiology and pathogenesis of KBD are still undecided. Several hypotheses have been proposed, including selenium deficiency and iodine deficiency in staple grains contaminated with fungal species (Li et al. 2008). MON caused chondrocyte necrosis, apoptosis (Zhao 2002), and DNA damage (Cao et al. 1995).

29.7.1 Mechanisms of Action

MON acts by inhibiting a number of enzymes that are dependent on thiamine pyrophosphate which in turn inhibits the process of gluconeogenesis (Pirrung et al. 1996). Apart from this, MON has also been reported to inhibit glutathione peroxidase and reductase (Chen et al. 1990).

29.8 Ochratoxins (OTs)

Ochratoxins are produced by several species of *Penicillium* and *Aspergillus,* which contaminate the diversity of foodstuff. OTA was first isolated in 1965 from a culture of *A. ochraceus* in South Africa (van der Merwe et al. 1965a). Three naturally occurring forms of ochratoxin are OTA, OTB, and OTC, but only OTA and OTB are most important in terms of effects on human and animal health. Among them, OTA

is most toxic than OTB and OTC (van der Merwe et al. 1965b). Ochratoxin A is mostly a contaminant of food grains and cereals, especially maize, oats, wheat barley, and other commodities such as cowpea, peanuts, dry foods such as dried and smoked fish, soybeans, garbanzo beans, nuts, dried fruit, cheese, and meat products of animals consuming ochratoxin-contaminated grains (Van Egmond and Speijers 1994; Aish et al. 2004), swine products (Van Egmond and Speijers 1994), beer (Scott and Lawrence 1996), and grapes and grape product including raisins, wines, and vinegars (Sage et al. 2004; O'Brien and Dietrich 2005). Wet milling of corn resulted in reductions of ochratoxin levels in germ and grits by 96% and 49%, respectively (Wood 1982). McKenzie et al. (1997) reported that ozonolysis is effective in destroying ochratoxin in aqueous model systems.

Ochratoxins are clinically considered as nephrotoxic, teratogenic, carcinogenic, genotoxic, and immunotoxic and has been classified by the IARC as a Class 2B carcinogen in human. OTA is a nephrotoxic causing fetal kidney disease in humans. This first occurred in 1956 in an epidemic form in the Balkan Peninsula region; thus, the disease is known as the Balkan endemic nephropathy (BEN) disease, which is characterized by hypercreatinemia, uremia, hypertension, edema (JECFA 2002; Mantle et al. 2010), acute renal failure, and acute tubular necrosis (Simon et al. 1996). Description of the clinical symptoms of OTA in humans is headaches, lumbar pain, anemia, proteinuria, and weight loss (Pfohl-Leszkowicz et al. 2002). Ochratoxicoses mainly affect poultry, rats, and pigs and lead to kidney damage, anorexia and weight loss, vomiting, high rectal temperature, conjunctivitis, dehydration, general weakening, and animal death within 2 weeks (Chu 1974). Ochratoxin damages the kidneys and liver and is also a suspected carcinogen. There is also evidence that it impairs the immune system. Chronic poisoning induces a decrease in ingestion, polydipsia, and kidney lesions. Pigs are particularly sensitive to OTA (Elling and Moller 1973). Such poisoning has a significant effect for toxin concentrations exceeding 1400 μg/Kg of feed. OTA has genotoxic properties due to DNA adduct formation (Pfohl-Leszkowicz 2000).

29.8.1 Mechanisms of Action

OTC and OTA have a chlorine group on carbon 5. The chlorine group is replaced with a hydroxyl group in some cytochrome P450 enzymes, creating OTQ, which undergoes redox cycling between a quinone and paraquinone, generating free radicals in the process (Josephy and Mannervik 2006). OTA makes kidneys more vulnerable to free radical damage and nephropathy. OTA seems to be a protein synthesis inhibitor by the inhibition of enzymes acting on phenylalanine metabolism, lipid peroxidation, and mitochondrial function (Creppy et al. 1983) and may cause DNA single-strand breakages with later stage genotoxicity and carcinogenicity (Pfohl-Leszkowicz and Manderville 2007).

29.9 Patulin (PAT)

Patulin ($C_7H_6O_4$, MW 154.12) is a polyketide which is produced by approximately 60 species of molds belonging to 30 fungal genera like *Penicillium*, *Aspergillus*, and *Byssochlamys,* among which the most important is *P. expansum* (Moss 1996; Steyn 1998; Leggott and Shephard 2001; D'Mello 2003; Gökmen et al. 2005). Apricots, grapes, peaches, pears, apples, olives, cereals, and low-acid fruit juices are the commodities most commonly infected by the patulin-producing pathogens (Moss 1996; Leggott and Shephard 2001; Speijers 2004). Apples and apple products are considered to be an extreme contributor to PAT in the diet. Contaminated apple juice usually contains PAT at levels below 50 ppb, but higher levels up to 4000 ppb have been reported intermittently (Drusch and Ragab 2003; Murphy et al. 2006). Acute effects of PAT include nausea, vomiting, and other gastrointestinal symptoms, which are convoyed by kidney damage (Speijers et al. 1988). A high-concentration PAT has immunotoxic properties. In a long-term study, PAT has been reported to be mutagenic and to cause neurotoxic, immunotoxic, genotoxic, and gastrointestinal effects in rodents (Hopkins 1993). PAT alters the intestinal barrier function (Mahfoud et al. 2002). Similar effects may occur in humans through prolonged consumption of foods and beverages contaminated by this mycotoxin. The IARC has classified PAT in Group 3 (IARC 1993a).

Bissessur et al. (2001) described that filtration to clarify juices and concentrates reduced PAT levels up to 40%. The level of PAT remains unstable during fermentation of apple juice to cider and is entirely destroyed by treatment with 0.125% sulfur dioxide (Lipowska 1990). Partially, PAT destruction was demonstrated by using pasteurization at high temperature in short time (Wheeler et al. 1987). In naturally contaminated apple juices, there is 25% loss in PAT when using pasteurization or under evaporation conditions (70–100 °C) as reported by Kadakal and Nas (2003).

29.9.1 Mechanisms of Action

PAT has been shown to induce cell death through inducing nonprotein SH depletion and altered ion permeability and communications leading to oxidative stress that eventually inhibits macromolecular biosynthesis in human cells (Riley and Showker 1991; Wouters and Speijers 1996). It may cause hemorrhage in the brain and lungs usually associated with fruit spoilage.

29.10 Sterigmatocystins (STs)

Sterigmatocystins (STs) are produced by different *Aspergillus* spp., viz., *Aspergillus versicolor, A. chevalieri,* and *A. ruber*, and other fungi such as *Penicillium, Bipolaris, Chaetomium,* and *Emericella*. They are extensively distributed in soil, peanuts, bread, sausage and cereal grains such as barley, rice, and maize (Jelinek et al. 1989). Sterigmatocystins are biogenic precursor compounds to AFB_1 and structurally

similar (Hamasaki and Hatsuda 1977); however, they are less toxic than aflatoxins (Sweeney and Dobson 1998). Hence, there is similarity in the potent carcinogenic, mutagenic, and teratogenic properties (Versilovskis and De Saeger 2010). Sterigmatocystin was also reported to have high effects on the liver, and esophageal cancers have been reported in Mozambique and China (Bhatnagar et al. 2002). Sterigmatocystins are of less importance than most of the other mycotoxins as far as human risk assessment is concerned (Versilovskis and De Saeger 2010).

29.10.1 Mechanisms of Action

The mode of action is suspected to be identical to aflatoxin because of the structural similarities between the two compounds: the mutagenic properties are most likely caused by the binding to the same N_7 guanine adduct of DNA molecules just like aflatoxin (Versilovskis and De Saeger 2010).

29.11 Trichothecenes (THs)

The trichothecenes are a family of more than 60 sesquiterpenoid metabolites, and they are produced by several genera of fungi, i.e., *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, *Trichothecium,* and some others. Deoxynivalenol (DON) or vomitoxin, diacetoxyscirpenol (DAS), nivalenol (NIV), and T-2 frequently contaminate foods and feeds. Both T-2 toxin and DON are most toxic to pigs and poultry (Friend et al. 1992) and was first found in barley and characterized in Japan (Yoshizawa and Morooka 1973). High incidence of TH was found in barley, corn, rye, wheat, oats, rice, safflower seeds, and mixed feeds worldwide (Visconti 2001; CAST 2003; D'Mello 2003). The biosynthetic pathway for TH production is known for several *Fusarium* spp. (Desjardins et al. 1993), and 11 genes have been cloned (Brown et al. 2001). In an incident in the Kashmir Valley of India, several thousands of people were affected by gastrointestinal distress in 1987. Symptoms included throat irritation (63%), diarrhea (39%), vomiting (7%), blood in the stools (5%), and facial rash (2%). Illnesses subsided when consumption of the bread ceased (Bhat et al. 1989). These people had feelings of fullness and mild to moderate abdominal pain within 15 min to 1 h after consuming their breakfast or evening snack. TH is also recognized as a strong immunosuppressive agent, as it can affect immune cells directly or by modifying immune responses (Sharma 1993). The immune stimulation generated due to mycotoxin poisoning may possibly lead to undesirable hypersensitive reactions (Sharma and Zeeman 1991). Immunosuppression is highly variable with mycotoxin offensive, which likely decreases and even predisposes the host to cancerous cell expression and disposition (Sharma 1993). The first toxicity action of DON is understood to be the modulation of the innate immune system. Pathological outcomes resulting from chronic low-dose exposure include anorexia, impaired weight gain, growth hormone imbalance, and anomalous IgA production; however, acute high-dose exposure induces

gastroenteritis, emesis, and a shock-like syndrome (Pestka 2010). Some common symptoms also include anemia, leukopenia, skin irritation, feed refusal, reduced growth, and reproductive failure (Whitlow et al. 2010). Major signs of T-2 toxicosis in pigs are gastrointestinal edema and hematopoiesis leading to death in suckling piglets (Hayes and Wobeser 1983; Glavitis and Vanyi 1995). T-2 toxin also has effects on lymphocytes specifically in the case of alimentary toxic aleukia (Taylor et al. 1989; WHO 1990). TH has been confirmed to induce chronic toxicity, genotoxicity, carcinogenicity, and immunotoxicity.

TH has been reported as a potential biological warfare agent. For instance, in an investigation of biological warfare agents in Cambodia from 1978 to 1981, T-2 toxin, DON, ZEN, nivalenol, and DAS were isolated from water and leaf samples collected from the affected areas (Watson et al. 1984). Partial to complete destruction of DON can be achieved with treatment of 10.0 pH aqueous buffer solution at 100 °C for 60 min and 120 °C for 30 min (Wolf and Bullerman 1998). Treatment of wheat and corn with dry and aqueous sulfite gas and ozone has also been investigated resulting in partial to complete destruction. DON levels in corn were reduced by as much as 95% by autoclaving at 121 °C for 1 h with 8.33% aqueous sodium bisulfite. DON levels above 1 ppm are not acceptable for use in products for human consumption (Murphy et al. 2006).

29.11.1 Mechanisms of Action

DON appears to affect serotonergic activity or serotonin receptors (Rotter et al. 1996). DON and T-2 toxin caused polyribosomal breakdown in mammalian cell lines (Ehrlich and Daigle 1987). THs are immunosuppressive, a strong inhibitor of protein synthesis, toxic to cell membranes, and induce apoptosis in various parts of the body (Sharma 1993; Yiannikouris and Jouany 2002). T-2 toxin has a strong affinity for the 60S ribosomal subunit and inhibits the activity of peptidyl transferase and, consequently, also protein synthesis in the initiation phase (Beasley 1989). The T-2 toxin reduces responses to mitogens in human lymphocytes in vitro (JECFA 2000).

29.12 Zearalenone (ZEA)

Zearalenone is a phytoestrogenic compound known as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid μ-lactone, Zearalenone (ZEA), or F-2 toxin; the name ZEA was given after its isolation from *Zea mays* infected with the fungus *Gibberella zea* (Marasas et al. 1980). It is mainly produced by *Fusarium* spp. including *F. graminearum*, *F. culmorum, F. equiseti*, and *F. crookwellense* (Bennett and Klich 2003). They colonize a wide variety of grains, including maize, sorghum, wheat, barley, oats, sesame seed, soybeans, and their derived foodstuffs (Bennett and Shotwell 1979; Kuiper-Goodman et al. 1987; Hagler et al. 2001). ZEA has been reported to occur in hay and silages in many areas of the world (Whitlow et al. 2010). The conditions that worsen ZEA accumulation in corn include moisture

contents at 22–25% or delayed harvesting (Abbas et al. 1988). Zearalenone is classified as an estrogenic mycotoxin because it gives estrogenic responses in animals and humans. Metabolites can be detected in blood plasma. ZEA is able to induce endometrial adenocarcinoma, endometrial hyperplasia, and normal proliferative endometrium (Tomaszewski et al. 1998; JECFA 2000). In addition, ZEA has been suspected to be the causative agent in an epidemic of precocious reproductive problems and physical changes in genital organs similar to those induced by estradiol: edemas and hypertrophy of the genital organs of prepubertal females, decrease in the rate of survival of embryos in gestating females, decrease in the amounts of luteinizing hormone (LH) and progesterone affecting the morphology of uterine tissues, decrease in milk production, feminization of young males due to decreased testosterone production, infertility, and perinatal morbidity (Saenz de Rodriguez 1984; Saenz de Rodriguez et al. 1985). Pigs are highly susceptible to ZEN poisoning in comparison to chickens and cattle (Coulombe 1993). ZEN is produced in very small amounts in natural conditions and probably in insufficient quantities to cause trouble in ruminants (Guerre et al. 2000). However, ZEN has been shown to cause infertility in grazing sheep in New Zealand (Towers and Sprosen 1993). Genotoxicity is reported to be of concern with respect to ZEN. This estrogenic compound showed chromosomal anomalies in some lymphocyte, oocyte, and kidney cell cultures when present within a range of 0.1–20 μM (Stopper et al. 2005). Zearalenone toxin is similar in chemical structure to the female sex hormone estrogen and targets the reproductive organs causing endometrial endocarcinoma disease. However, it can cause estrogenization and pseudopregnancy in women and inhibition of the development of testicles in men (Resanovic et al. 2013).

29.12.1 Mechanisms of Action

Zearalenone and its metabolites have been shown to competitively bind to estrogen receptors in a number of in vitro systems. Binding to specific receptors has been demonstrated in uterus, mammary gland, liver, and hypothalamus from different spp. (Eriksen and Alexander 1998; JECFA 2000). α-Zearalenol, which has about threefold more estrogenic potency than zearalenone, was recently shown to inhibit atherogenesis, lowering plasma LDL-cholesterol and limiting aortic plaque formation in ovariectomized rabbits fed on a high dose of cholesterol. The effective estrogenic dose of α-zearalenol was >0.5 mg/Kg/d for 12 weeks (Dai et al. 2004).

29.13 Ustiloxins

The recently discovered ustiloxins are cyclopeptide mycotoxin produced by *Ustilaginoidea virens*. Ustiloxins A–E were illustrated and identified from false-smutted balls of rice and their products infected by pathogen, *Ustilaginoidea virens* Cook Takahashi (Koiso et al. 1994). It has been reported that the infected rice plant causes poisoning to animals. Ustiloxins A and B are more toxic than types C, D, and

E in terms of effects on vertebrates. Ustilotoxin A caused lesions in the liver and kidney in mice similar to lupinosis caused by phomopsin A. Isolation of the toxic substance demonstrated that the contaminated rice panicles may cause toxicosis of cattle (Nakamura et al. 1994). The fungus produces ustiloxin, mycotoxin, and phytotoxin that contaminate the rice grains and straws. Ustiloxin is an antimitotic cyclic peptide (Luduena et al. 1994).

29.13.1 Mechanism of Action

Ustiloxins strongly inhibit the polymerization of microtubule activities. Ustiloxin A is the most potent inhibitor of tubulin polymerization currently known (Li et al. 1995). Ustiloxins A and B inhibited the mitosis of human tumor cell line and also caused liver and kidney lesions in mice (Koiso et al. 1994).

29.14 Mycotoxins and Fungal Growth Affecting Factors

Extrinsic factors such as water, temperature, pH, and gas composition have a profound influence on the growth of mycotoxin-producing fungi and mycotoxin biosynthesis. Mycotoxin presence in raw and stored feed materials is affected by environmental factors. Mycotoxin biohazard of foods has been endeavored through control of water activity, pH, and quality control measures. Thus, knowledge of these physio-biochemical parameters can provide methodology for preventing fungal growth and mycotoxin production in foods and animal feeds. However, it should be pointed out that interactions among several of these factors are often more important than individual factors acting in isolation (Smith 1994). These factors are as following.

29.14.1 Influence of Temperature

Widstrom (1992) described the infection and colonization period of corn by *A. flavus* between silking at about 60 days after planting until physiological maturity at about 120 days. There was increasing damage to the ear by insects from silking to harvest at 135 days after planting. There was a continuous post-infection aflatoxin accumulation from silking to harvest. The optimal temperature (25–32 °C) for corn production is lower than the optimal temperature (35–37 °C) for *A. flavus* growth and insect damage. Consequently, there are positive correlations between the average temperature, aflatoxin contamination, and insect damage. This is especially evident when temperatures become too high for optimum corn growth. Ears that are developing and maturing when the temperature is 28–32 °C are much more susceptible to aflatoxin contamination than ears that develop at lower temperatures when the corn is planted later.

Penicillium spp. require a narrower temperature range (25–30 °C) for growth than *Aspergillus* spp. (15–40 °C), whereas the optimal temperature range of 28–30 °C is ideal for *Penicillium* and 37–47 °C for most *Aspergillus* (Pitt and Hocking 1997). *Fusarium* spp. are capable of growth and reproduction in cold temperatures, i.e., psychrophilic in nature (Robert and Raymond 1994), although the temperature required for the optimal production of most mycotoxins range between 25 °C and 33 °C according to the type of fungal species and the type of mycotoxins produced (Pitt and Hocking 2009). The optimum growth of *F. verticillioides* was at 25–30 °C temperature and the optimum temperature for FB1 production was 25 °C (Torres et al. 1998; Fodor et al. 2006). The maximum production of OTA was obtained at 30 °C by *A. ochraceus* (Abarca et al. 2003).

29.14.2 Influence of Humidity

In agricultural practice, safe water content, usually expressed as percent moisture, has been established, for the storage of particular commodities. Because the water activity (a_w) is greatly influenced by the type and quantity of soluble solids present, safe water contents vary from food to food and even from region to region. The safe water content of peanuts is about 8%, of soybeans 12.5%, of cereal grains 13.5–14.5%, of polished rice 15%, and of Australian prunes as high as 23% (Pitt 1965; Christensen 1978). Fast-growing xerophiles such as *Aspergillus* species compete with other diverse conidial fungi over almost the entire range of water availability (Cole 2012). Marques et al. (2009) evaluated the incidence of *Aspergillus*, *Fusarium,* and *Penicillium* fungi and the contaminations with mycotoxins in grains of five corn commercial hybrids due to harvest humidity. The genus *Fusarium* presented linear increasing incidence due to increasing humidity, whereas the incidence of *Aspergillus* spp. and *Penicillium* spp. decreased.

29.14.3 Influence of A_w

Water availability is usually expressed as equilibrium relative humidity (ERH), water activity (a_w), or water potential (Ψ). Scott (1957) first introduced the concept of a_w, which is widely used in the food industry as a measure of water availability for microbes. In each case, the safe water content corresponds to 0.70 a_w. The fungi involved in spoilage or deterioration of the types outlined above must by definition be xerophiles. A key to xerophile genera is given by Pitt (1975). The types of fungi involved in spoilage and deterioration above the boundary line of 0.85 a_w are much less clearly defined.

Aspergillus species differ in their a_w requirements, and therefore, the presence of a particular species in a food or feed is often a good indicator of the previous storage conditions. *Eurotium* species, better known as members of the *A. glaucus* species group, are the most xerophilic group of fungi, capable of growing at the lowest

limits of a_w, namely, 0.71 a_w. *Aspergillus restrictus* and *A. penicillioides* are also considered to be xerophilic. *Aspergillus candidus* and *A. ochraceus* require 0.75 a_w, *A. versicolor* and *A. flavus* require 0.78 a_w, and *A. fumigatus* require 0.75 a_w and 29 °C. Similarly, at less than 0.75 a_w, conidia remained dormant but viable (Teitell 1958). With decreasing a_w, lag time before germination increases. At high water activities (>0.98 a_w), lag time can range from a few hours to several days, while at low a_w, this can extend to several months. At 0.62 a_w, *A. brunneus* spores took 730 days to germinate, producing abnormal germ tubes but no mycelial growth (Snow 1949). Germ tube elongates rapidly at high a_w until 150–250 μm in length (Smith 1994).

Aspergillus spp. and *Penicillium* spp. are the most xerotolerant group of fungi. However, *Penicillium* spp. are less well-adapted to low water activities when compared with *Aspergillus* spp. The lowest water activities will permit germination of *Penicillium* spp. in culture and lie in the range 0.78–0.84 a_w, compared with below 0.78 a_w for the majority of *Aspergillus* spp. (Cole 2012; Neville 2012). In temperate climates, xerotolerant *Penicillium* spp. are common compared to *Aspergillus* spp. in soil and tend to replace them on substrata with higher moisture contents. In general, *Aspergillus* spp. can only compete successfully with *Penicillium* spp. at higher temperatures and low water activity (Magan and Lacey 1984).

If the moisture content of the stored materials remains at a minimum of about 12% of the fresh weight; the growth of storage fungi will be prevented. The corresponding water activity for non-oily grains stored at this moisture content is below 0.60a_w, but sunflower seeds, groundnuts, and other oily seeds need to be stored somewhat drier because of their corresponding higher water activity. If, however, storage conditions are bad and the moisture content rises or the materials have moisture content above the critical 12% when placed in store, contaminating storage fungi will slowly colonize (Clarke et al. 1967; Neville 2012). While most storage fungi grow at a_w below 0.75 generally, the required a_w for fungal growth is between 0.61 and 0.91 (Robert and Raymond 1994). *Penicillium* and *Aspergillus* grow best at a_w of 0.95, whereas *Fusarium* grow best at higher a_w of 0.98 (Smith and Moss 1985; Moss 1996). High moisture in the field and at storage may determine the extent of mycotoxin contamination in stored food and feed (Hussein and Brasel 2001). Aflatoxin production was highest at 0.98 and 0.95 a_w at 25 °C, ZEA at 0.98 a_w at 25 °C and 0.95 a_w at 16 °C, and the production of OTA was best at 25 °C (Bhat et al. 2010).

29.14.4 Influence of Oxygen Tension

The most important and essential element required for fungal growth is O_2, but certain species can also grow under anaerobic conditions with the formation of ethanol and organic acids (Northolt et al. 1979). However, the absence of oxygen is an important factor in controlling spoilage in many kinds of foods, e.g., grain in silos, packaged meat, canned fruit, and bottled beverages. Most fungi require at least

1–2% O_2 for growth (Tuite et al. 1985), while mycotoxin production can also be influenced by the presence or absence of O_2 in the environment (Pitt and Hocking 2009). Most *Aspergillus* spp. growth is inhibited at <1% O_2 concentration (Pitt and Hocking 1997). *Fusarium verticillioides* is an exception, as it is able to grow at 60% CO_2 and less than 0.5% O_2 (Tuite et al. 1985; Keller et al. 1997).

In atmospheres containing reduced oxygen concentrations, Golding (1940a, 1945) reported that growth of *P. roqueforti*, *P. expansum, Aspergillus flavus, A. niger,* and *Geotrichum candidum* was little affected even at <4.2% of oxygen content. In similar experiments, Golding (1940b, 1945) also studied the influence of carbon dioxide on fungal growth. He showed that the growth of the species mentioned above was stimulated by increase in the carbon dioxide concentration up to 15% in air but that further increase caused a decline in growth rates.

29.14.5 Influence of CO_2

High carbon dioxide levels produced by respiring grain in airtight storage can prevent mold growth, but such levels are not readily attainable in commercial practice. Carbon dioxide levels must be very high to prevent positively the growth of some conidial fungi. Nevertheless, Peterson et al. (1956) reported that even in the presence of 21% oxygen, 13–18% carbon dioxide markedly improved the quality of wheat stored at 18% moisture (0.85 a_w). Clearly, the effect of carbon dioxide is enhanced by the reduced a_w of storage. On the other hand, Peterson et al. (1956) found that a reduction in oxygen levels down to 0.2% (in nitrogen) did not completely suppress mold growth.

Zhai et al. (2015) showed that the CO_2 concentrations increased exponentially ($r >= 0.96$) during the growth of the toxigenic fungi *Aspergillus flavus*, *Penicillium* sp., and *Aspergillus ochraceus*, which was different from the linear increase of CO_2 concentration produced by the nontoxigenic xerophilic fungi *Aspergillus glaucus* and *Aspergillus restrictus*. The acceleration of CO_2 concentration was found much earlier than the growth of toxigenic fungi, which would be useful for the prevention of grain spoilage. The results can provide a valid foundation for the prevention of toxigenic fungi and mycotoxin production in stored grains through monitoring the CO_2 concentration changes.

29.14.6 Influence of pH

The H^+ ion concentration has also been found to be an important element in the production of mycotoxins. Most bacteria prefer neutral pH (6.5–7.5); molds and yeast grow in wider pH range but prefer pH between 5.0 and 6.0, reflecting the general effect of change $[H^+]$ on the rates of enzymatic reaction. There are neutrophils (pH 5.4–8.0), acidophiles (pH 0.1–5.4), and alkalinophiles (pH 8.5–11.0) based on the pH of the habitat of an organism; and any change affects the population because

strong acids and bases can be highly damaging to enzymes and other cellular substances (Brock 1986; Talaro 2005). Yamanaka (2003) highlighted that many of the saprotrophic fungal species grew well at pH 7 or 8.

The optimum range of pH value necessary for active growth of *A. ochraceus* is 3–10 pH; for fungi from a section of *Aspergillus niger,* the optimum pH value is 4.0–6.5. *Aspergillus carbonarius* species is able to grow and produce mycotoxins at a wider range of pH values from 2 to 10 (Pitt and Hocking 2009; Grigoryan and Hakobyan 2015). As seen, optimum pH for AF production by *Aspergillus* spp. is between 3.5 and 8.0 (ICMSF 1996), while OTA production by *A. ochraceus* is minimum at a pH value of 2.2 (Wheeler et al. 1991) and that for FB1 production by *F. verticillioides* is at a pH value of 7.5 (Narasimha Rao et al. 2010). The effect of pH seems to vary with the medium composition as Keller et al. (1997) found that at a pH between 3.0 and 4.0 in liquid culture, optimal FB1 production was observed.

29.15 Management

29.15.1 Preharvest Strategies

- Maintenance of proper planting/growing conditions, i.e., soil testing, field conditioning, crop rotation, and irrigation.
- Use of antifungal organic acids treatments, i.e., acetic acid (Buchanan and Ayres 1979), propionic acid and butyric acid (Ghosh and Haggblom 1985), malonic acid (Megalla and Hafez 1982), benzoic acid (Chipley and Uraih 1980; Uraih and Offonre 1981), sorbic acid (Yousef and Marth 1984), lactic acid, citric acid (Reiss 1979), and their sodium salts to reduce the fungal infection.
- Adequate insect and weed prevention.

29.15.2 Harvesting Strategies

- Use of functional harvesting equipment, clean and dry collection/transportation equipment.
- Appropriate harvesting conditions, i.e., low moisture and full maturity (<9% for peanut kernel and < 13.5% for corn (FAO 1979) and other cereals).
- Removal of contaminated seeds.
- Use of drying as dictated by moisture content of the harvested grain, appropriate storage conditions.
- Complete destruction of the contaminated products.
- Use of transport vehicles that are dry and free of visible fungal growth (Quillien 2002; CAC 2003).
- Gamma irradiation (5–10 M-rad) caused reduction of aflatoxin (Sommer and Fortlage 1969).

- Benzoic acid derivatives: Onitrobenzoate, O-aminobenzoate, paminobenzoate, benzocain (ethyl aminobenzoate), ethyl benzoate, methyl benzoate, and aspirin (O-acetoxy benzoic acid) prevent growth and invasion of pathogenic fungi in agricultural commodities (Davis and Diener 1967).
- Fumigant: Ammonia and phosphine to inhibit growth and invasion of pathogenic fungi (Vandergraft et al. 1975).
- Approach involves increasing production of compounds, i.e., antifungal proteins or secondary metabolites, such as hydroxamic acids, phenolics, and stilbenes, to reduce infection by the microorganism.
- Selection of fungal resistant hybrids of crops is recommended.
- Use of Bt hybrid reduced mycotoxin production compared to the parent corn (Munkvold 2003).
- Representative sampling and effective diagnostic tools that can be used to monitor and quantify mycotoxins rapidly (Magan and Aldred 2007).

29.16 Conclusion

The seeds are excellent media for the growth of fungi, and therefore, a very high standard of hygiene is necessary to avoid seed contamination. The high occurrence of fungal species of public health concern may indicate obvious health hazards in terms of direct consumption of fungus-contaminated food/feed or their mycotoxins by farmed animal and subsequent public health problem. Due to this fact, regular microbiological and also myco-toxicological analysis is necessary for the determination of quality and safety of livestock feed. Contaminants of foods and feeds with mycotoxins are a worldwide serious problem. The most important mycotoxins in terms of toxic effect on both humans and animals are aflatoxins (AFs), citrinin (CIT), cyclopiazonic acid (CPA), fumonisins (FBs), moniliformin (MON), ochratoxin A (OTA), deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), patulin (PAT), zearalenone (ZEA), and ustiloxins. These mycotoxins contaminate food and feeds and pose numerous adverse effects or death in both human and animal. Toxic effect of mycotoxins is as carcinogenic, immune suppressive, nephrotoxic, neurotoxic, mutagenic, cytotoxic, and estrogenic both in humans and animals. Pre- and postharvest management strategies are most important for the management of toxicogenic fungi in food materials. Use of mycotoxin-detoxifying agents can suppress or reduce the absorption, promote the excretion of mycotoxins, or modify their mode of action. These feed additives transform mycotoxins into less toxic metabolites either by reducing their bioavailability or by degrading them. These can be defined at least into two main categories including first various mycotoxin-adsorbing agents and second bio-transforming agents that lead to the degradation of mycotoxins into nontoxic metabolites.

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Epidemiology of Seed-Borne Diseases

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Abstract

Epidemiological information is the key for considering control strategies specific for seed-borne diseases. For seed-borne pathogens, it is important to understand how pathogens behave in particular environmental conditions which may be useful to develop a cost-effective control measure in the present climate change scenario. The behavioral knowledge of pathogens may be utilized for creating unfavourable environmental conditions for avoiding infection by seed treatment (biological, botanical or chemical) or growing the crop in disease-free areas or unfavourable environments to avoid crop loss. Moreover, to develop effective, sensitive and accurate seed testing techniques, epidemiological issues like quantity of potential inoculums, risk of seed-borne inoculums, relation between seed health and disease and environmental factors affecting seed-to-seedling transmission during seed germination to crop growth are important aspects which have been described in this chapter.

30.1 Introduction

Epidemiology deals with the study of pathogen population in the population of host and the diseases resulting therefrom under the influence of environmental and human interference (Kranz 1990). Epidemiological information about inoculum thresholds of seed-borne diseases must be available before considering control strategies for specific seed-borne diseases. For seed-borne pathogens, inoculum

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threshold is the level of infection on or in seed that significantly affects disease development and results in economic loss (Gabrielson 1988). Therefore, epidemiological classification of seed-borne diseases into external and internal infestation of seed is important. Seed-borne diseases based on the infection site may be called as either internally or externally seed-borne disease. Although initial infection is from the seed, however in the field pathogen spread may occur through air or soil or even water. Based on the spread they may be monocyclic or polycyclic. Spread through soil or water is mostly of monocyclic nature and otherwise called as soilborne, thus specifying the ecological nature of disease. Disease spread by air is typically polycyclic and is called as airborne disease. Thus, the epidemiology of seed-borne diseases is a complex interaction between host, pathogen and environment as well as human interference. The amount of seed infection at or during the harvest is dependent on the interaction.

30.2 Factor Effecting Seed-Borne Pathogens

30.2.1 Quantity of Potential Inoculums in or on Seeds

Most of the seed-borne pathogens are carried on seed surface; they may survive under the seed coat or as a contamination to seeds. Some seed-borne pathogens can survive inside the seed in embryo or in ovule (virus) or in transplants inside the apoplast or up to vascular tissue also (bacteria, phytoplasmas and viruses) (Agrios 2005). Most pathogens, which are distributed worldwide, were permitted in small amount of inoculums, but with the development of races of these pathogens, these are taken into serious consideration. These races when acclimatize to a new environment leads to disastrous to specific crops, varieties, cultivars or new hosts (*Magnaporthe oryzae*, *Melampsora lini*, *Phytophthora infestans*, etc.) (Neergaard 1969). Successful transmission of seed-borne pathogens depends upon infection location or presence of potential inoculums in or on seed. Theoretically, *Tilletia tritici* can cause infection with a single spore, but a minimum population of 40–100 spores/seed was reported to cause successful infection in susceptible varieties (Heald 1921; Oxley and Cockerell 1996). Similarly, bacteria also produce symptoms when they reach to 10^6 cells (Agrios 2005). The amount of inoculum on the seed and disease transmission are usually strongly influenced by environmental conditions and cultivar susceptibility.

30.2.2 Level of Virulence and Nature of Disease (Monocyclic or Polycyclic) Caused by Seed-Borne Pathogen

Some pathogens are less virulent in particular situations because of change in agronomical practice or introduction in a new environment. *P. infestans* survives in potato seed tubers in dormant condition; it becomes more virulent in Europe than in South America because of high temperature (Agrios 2005). *Ustilago nuda* and

U. tritici cause loose smut in barley and wheat, where no spread occurs between plants during crop growth. The relationship between seed infection and plant infection is relatively constant, and it is only at flowering, when seed re-infection takes place, environmental conditions influence disease development. Cool moist conditions at flowering can lead to higher rates of re-infection because the flowers remain open for a longer period (Hewett 1978).

With monocyclic diseases, reducing or eliminating seed-borne inoculum (through appropriate seed treatment) usually gives effective disease control. Polycyclic diseases, on the other hand, once established in a crop, will multiply and spread under suitable conditions. They frequently cause local lesions on infected plants, and as the disease progresses, these lesions increase in size and produce increasing quantities of inoculum, which spread to other parts of the plant, and to neighbouring plants. Here, the relationship between seed-borne inoculum and plant infection is complex. Environmental conditions, inoculum from other sources, including volunteer plants and old crop debris, complicate the seed-to-plant disease relationship (Rennie and Cockerell 2006).

30.2.3 Environment Factors in Seed-to-Seedling Transmission

Various environmental factors are involved in successful transmission of seed-borne pathogen to establish plant infection and cause diseases during germination and seedling establishment. Loose smut in wheat is mostly present inside the seed embryo. If infectious seed is planted in cool and moist environment, the mycelium becomes active, and it grows along with the plant and reaches the ovaries which are transformed into black mass of teliospores (Neergaard 1969). If there is a delay in sowing, the pathogen cannot establish successfully leading to decrease in severity of disease (Singh et al. 2009).

At low soil water condition, transmission of some of the seed-borne (caused by lower fungi) diseases is reduced, but at high soil moisture it causes devastating effect on crop (Rennie and Cockerell 2006). On the other hand, where inoculum is deep-seated and the embryonic axis of the seed is infected, environmental factors may have relatively little effect on pathogen transmission. *Ustilago nuda* in barley is established in the scutellum tissues of the seed embryo and is likely to give rise to plants showing symptoms over a wide range of environmental conditions. It is greatly reduced in dry seedbeds (Hollaway et al. 1996).

30.2.4 Various Other Factors for Establishment of Infection and Spread of Pathogens

Some seed-borne pathogens are systemic in nature (Downy mildew) with the capacity to infect crop at any growth stage, these pathogens have very little or no influence of environmental condition on the seed infection rate. Those seed-borne pathogens, which also have soil- or air-borne mechanism, have very little or no

influence on the seed infection rate. Bacterial and fungal populations often develop very rapidly on diseased plants in humid or wet conditions, and seed infection usually depends on the availability of adequate moisture. For many diseases, therefore, seed infection levels are highest when high humidity or rainfall occurs during seed development (Rennie and Cockerell 2006).

Seed-borne viruses can spread through insects also, so for such kind of relationship, the environment can affect both insect population and virus transmission. *Tomato leaf curl virus* requires low humidity and rainfall, because at high humidity and rainfall condition, white fly population is reduced drastically (Naveed et al. 2015)

Commercial seed production of various crops is undertaken in Hyderabad (India) because of dry conditions that lead to low incidence of various diseases in various crops. Seed production in areas of low disease pressure or in environmental conditions unfavourable for seed infection is a very effective means of producing healthy seeds and is likely to be most cost-effective when seeds are small and transport costs low, or when the seed is of sufficiently high value to justify high transport costs. With low-value, high-volume cereal seed, isolated seed production may not be practical or economic, and healthy crops may become infected during seed production if high levels of inoculum are present in neighbouring crops (Rennie and Cockerell 2006).

30.2.5 Quantity of Potential Inoculums or Inoculums Threshold

Most of the seed-borne pathogens are carried on seed surface or inside the seed and distributed worldwide through small amount of inoculums (Neergaard 1969). For successful seed-borne pathogens, transmission depends upon infection location or presence of potential inoculums in or on seed. Therefore, inoculum threshold for seed-borne diseases is an important component to develop cost-effective management strategy in present climate scenario. Seed infection is common, and it is impossible to completely eliminate risk. Therefore, we need to define what acceptable or unacceptable risks are and devise effective methods for determining these risks.

For seed-borne pathogens, inoculum threshold is defined as the amount of seed infection or infestation with plant pathogens that will cause a disease in the field under a conducive environment and lead to economic losses (Kuan 1988).

Based on Van der Plank's infection rate equation (r) for *Pseudomonas syringae* pv. *phaseolicola*, tolerant levels were determined (Taylor 1970):

$$r = \frac{2.3}{t_2 - t_1}\left[\frac{\log_{10} X_2}{1 - X_2} - \frac{\log_{10} X_1}{1 - X_1}\right]$$

It was found that an infection rate of 0.15 and a transmission rate of 10:1 were common in England for beans. Therefore, if infection level is 0.025% in a seed lot,

it won't lead to disease in the field condition. So, 4% infection at harvest level in green bean crop is considered to be a tolerant level.

Similarly, if primary infection was 0.01% and 0.006%, it leads to severe crop loss (Guthrie et al. 1965; Wharton 1967). This would represent seed infection level of 0.1% and 0.06% when assuming a seed transmission of 10:1, a level higher than the suggested tolerance level of 0.025%. This result agrees favourably with a tolerance of 0.01% for *Xanthomonas campestris* pv. *campestris* (Schaad et al. 1980).

30.3 Risk of Seed-Borne Inoculum

To define the risks from seed-borne disease, there is a need to quantify the relationship between disease in the crop and amount of pathogen in the seed. This requires quantitative data on transmission of the pathogen from seed to seedling and the rate of spread or increase of disease in the crop. To define unacceptable levels of disease in a crop whether for certification or quarantine purposes, there is a need to explain what could be an acceptable risk for such an unacceptable level of disease. The relationship between the inoculum dose on individual seeds and the transmission of the pathogen or appearance of primary infections in a crop can be interpreted in terms of the 'one-hit' infection model (Roberts et al. 1996). This model assumes that (1) each pathogen cell or propagule acts independently (i.e. each cell is inherently capable of causing infection); (2) the probability, w, of an individual being effective (i.e. giving rise to infection or transmission) is the same for all cells; (3) the host subjects are homogeneous; and (4) the potential number of infection sites is large. The probability, p, of an infested seed giving rise to an infected plant is:

$$p = 1 - \exp\left(-w.d\right)$$

where, w is the probability of infection for a single cell in the dose d. We can rearrange this equation to give:

$$\ln\left[-\ln\left(1-p\right)\right] = \ln\left(w\right) + \ln\left(d\right)$$

so that, theoretically, a plot of $\ln[-\ln(1-p)]$ vs $\ln(d)$ should have a slope of one and an intercept of $\ln(w)$. Unfortunately, real life doesn't always seem to fit the theory, and we have found it necessary to include an additional parameter, x, in the model:

$$p = 1 - \exp\left(-w.d^{x}\right)$$

Possible explanations of the need for this additional parameter are considered by Roberts et al. (1996), but essentially this extra parameter means that as the dose per seed increases, the effectiveness of individuals in that dose appears to decrease. Fitting the model to some of the few examples of published data yields the parameters shown in Table 30.1.

Relationship between inoculum thresholds, seed health assay efficiencies, and the calculation of statistically sound seed sample sizes are available for seed-borne fungi (Geng et al. 1983; Kuan 1988; Russell 1988; Schaad 1988; Roberts et al. 1993).

Table 30.1 'One-hit' model parameters for seed transmission for various host/pathogen combinations (Roberts et al. 1996)

Host/pathogen	*w*	*x*
Wheat/*Tilletia caries*	0.0006	0.76
Safflower rust/*Puccinia carthami*	0.010	0.62
Beans/*Pseudomonas savastanoi* pv. *phaseolicola*	0.054	0.18
Brassicas/*Xanthomonas campestris* pv. *campestris*	0.014	0.32
Peas/*Pseudomonas syringae* pv. *pisi* (wet soil)	0.063	0.24
Peas/*Pseudomonas syringae* pv. *pisi* (dry soil)	0.006	0.24

In the case of black rot-infected seedlings, Roberts et al. (1999) demonstrated that expression of disease and spread of the pathogen in the greenhouse were highly dependent upon the watering regime and initial inoculum density. The percentage of plants with black rot symptoms was significantly higher for plants frequently irrigated by overhead watering and planted from seed with 3.6×10^3 Xcc CFU/seed than for plants receiving less frequent irrigation, with a capillary style irrigation and planted from seed with 1.5×10^2 Xcc CFU/seed (Roberts et al. 1999). A predictive model was developed based on the proportion of plants with symptoms, the mean density of bacteria per seed and the number of overhead waterings. Generalised linear models were fitted to the data relating the proportion of symptomless contaminated plants or the proportion of plants with symptoms, p, to the mean dose of bacteria per seed, d, and the number of overhead waterings, *noh*.

The equations were:

$$p = 1 - \exp\left(-0.014 \cdot d^{0.32} \cdot \text{noh}^{0.045}\right) \text{ for symptomless contaminated / infected plants}$$

and

$$p = 1 - \exp\left(-0.0056 \cdot d^{0.44} \cdot \text{noh}^{0.014}\right) \text{for plants with symptoms}$$

These models indicated that the one-hit probability for transmission of the pathogen (i.e. with/without visible symptoms) was 0.014 and for infection (i.e. with visible symptoms) was 0.0056.

Further, effects of inoculum dose, inoculation method, temperature and soil water potential on transmission of bacterial blight (*Pseudomonas syringae* pv. *pisi*) from seed to seedling of pea were explained by Roberts et al. (1996). Dose received (mean number of bacteria per seed) and soil water potential had the greatest effects, with the proportion of plants infected and the mean number of lesions increasing with dose received and soil moisture. Inoculation method had little direct effect on transmission but greatly influenced the dose received. Temperature, in the range 5–18 °C, had no effect on the number of lesions and little effect on the proportion of plants infected. Generalised linear models were derived relating the proportion of plants infected, p, and the mean number of lesions, m, to the dose received, d, and the soil water potential, ψ.

The equations were:

$$p = 1 - \exp\left[-0.064\, d^{0.24} \exp\left(58.5\psi\right)\right]$$
$$m = 0.24\, d^{0.25} \exp\left(93.3\psi\right).$$

The exponents for dose in these equations were considerably less than the theoretical value of one implied by a one-hit model and suggested considerable heterogeneity in the one-hit probability.

30.4 Viral Diseases

For seed-borne viruses, inoculum could be tolerated depending on the type of virus infection in crops (Stace-Smith and Hamilton 1988). Viruses meeting these criteria would have an inoculum threshold of zero or close to zero having the following characteristics:

- If vector transmission is efficient, the level of seed transmission does not have to be high. It can be exceedingly low and still be of critical importance when a few infected seedlings arising from contaminated seed constitute the sole source of inoculum and when the virus is ready to be acquired and actively vectored in a crop.
- The crop must be annual, because infected seed usually would not be the sole inoculum source in perennial crops like potyvirus or cucumovirus group.
- The vector is an aphid and virus is transmitted in a non-persistent manner.

Otherwise, seed-borne viruses that are not as actively vector transmitted but may constitute the sole source of inoculum are epidemiologically important, and although tolerable levels are not generally stated, it is obvious that levels must be low. For those seed-borne viruses that have a broad host range, including both annual and perennial crops, seed transmission may play an insignificant role in virus epidemiology, and consequently, high inoculum levels could be tolerated.

30.5 Correlations Between Seed Health Assays and Disease

There are relatively few examples in the literature where there have been definitive experiments examining the relationship between seed-borne disease levels and disease in the field. Most examples relate to fungal pathogens and only a few to bacteria and viruses. In most cases the seed test results are reported in terms of the per cent of seeds infested, and in general the per cent of disease incidence correlates well with seed test results. There are a number of examples in the literature of good correlations between seed health test results and disease transmission. However, these data have often been obtained in one environment with one seed lot and with limited numbers of seeds sown in each plot. As a result, ‘tolerance standards’ based directly on such experimental results may be artefacts of the experiments themselves.

Most seed tests estimate seed infection in terms of the percentage of seeds infected, and the inoculum dose per seed has largely been ignored. However, there is clear data in the literature to demonstrate that inoculum dose has a major influence on disease or pathogen transmission. The environment into which the seed is sown can also have a major influence on transmission. The parameters of the models fitted to experimentally determine relationships between inoculum dose and transmission imply that the distribution of inoculum may play a critical role in determining the probability of transmission.

It is clear that more information on the distribution of inoculum on individual seeds is needed. There is also a need for models which integrate the apparent per cent infection and the mean dose per seed and to examine sensitivity of seed health tests in relation to inoculum dose as well as per cent infection, if we are to design more effective seed health assays and minimise the risk of disease.

30.6 Conclusion

In present situation demand of seed has increased enormously, creating huge pressure on the country to relax or remove barriers in seed trade. So, the major challenge in the future is to encourage and facilitate trade in seeds; on the other hand insurances of healthy seed movement is the biggest task. There should be a restriction on pathogens which pose greater threats to crop production. Seed production, especially of high-value seed, such as hybrid and genetically modified varieties, will be concentrated in countries, or regions, where specific seed-borne pathogens are absent, or which, because of climatic conditions, are conducive to healthy seed multiplication. There is an urgent need to study the ecology and biology of various seed-borne diseases in relation to climate change. Simulation model for various seed-borne diseases can help for development of effective management as well as evaluation of genotypes under climate change scenario. There has also been progress in understanding infection thresholds and how they influence seed sample size determination and ultimately the reliability of seed health testing. However, prediction of disease development and dissemination of pathogens from contaminated seed lots taking account of inoculum density and environmental pressures would make seed health testing more reliable.

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